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VOL. 17, SEC. C. MAY, 1939 NUMBER 5

SOME *PEZICULA* SPECIES AND THEIR CONIDIAL STAGES[1]

BY J. WALTON GROVES[2]

Abstract

The following species of *Pezicula* have been cultured and the genetic connection of their conidial stages has been established; *Pezicula carpinea* (Pers.) Tul. with the conidial stage *Cryptosporiopsis fasciculata* (Tode) Petr., *Pezicula pruinosa* Farl. with the conidial stage *Sphaeronema pruinosum* Peck, *Pezicula Corni* with the conidial stage *Cryptosporiopsis cornina* (Peck) Petr. and Syd., *Pezicula Rubi* (Lib.) Niessl with the conidial stage *Discosporiella phaeosora* (Sacc.) Petr., and *Pezicula Hamamelidis* n. sp. with the conidial stage a *Cryptosporiopsis* species. The form of the conidial spore is relatively constant in the different species, but the form of the conidial fruiting body is variable.

Introduction

It has been observed by mycologists from time to time that morphological correlations may exist between the perfect and imperfect stages of pleomorphic fungi, and, where such correlations do exist, they are of value in indicating relationships. The purpose of this paper is to describe, in some detail, certain species of the genus *Pezicula* and their conidial stages. The genetic connections have been established by cultural methods, and the conidial stages have proved to be of interest in that, in the different species, the form of the conidial spore remains relatively constant while the form of the fruiting body may be quite variable. The species to be discussed in this paper have been chosen for the purpose of illustrating the range of variation that has been found.

The genus *Pezicula* was erected by Tulasne (21), based on *Peziza carpinea* Pers., which he transferred from *Dermatea* Fries. In his account, Tulasne drew attention to the form of the conidia, which were stated to be ovate to oblong; and he described three other species, *Pezicula Coryli* Tul., *P. amoena* Tul., and *P. dissepta* Tul., which were also transferred from *Dermatea* where he had referred them in an earlier paper (20). The close relationship of *Dermatea* and *Pezicula* has been generally recognized by later authors, some of whom have not recognized *Pezicula* as distinct, but no attempt will be made here to trace the various generic concepts.

Pezicula species are typically bright coloured, waxy-fleshy in consistency, with rather broad asci, oblong-ellipsoid ascospores, and oblong-ellipsoid

[1] *Contribution No. 579, Botany and Plant Pathology, Science Service, Department of Agriculture, Ottawa, Canada. Most of the work was done at the Department of Botany, University of Toronto, Toronto, Canada, and the material constituted part of a thesis presented to the School of Graduate Studies of the University of Toronto in partial fulfilment of the requirements for the degree of Doctor of Philosophy.*

[2] *Graduate Assistant, Central Laboratory, Ottawa.*

conidia; while *Dermatea* species are typically dark coloured, more leathery in consistency, usually with relatively narrower asci, more fusiform-elliptical ascospores, and elongate to sub-filiform conidia. It has been pointed out by Groves (6) that exceptional species such as *Dermatea acerina* (Peck) Rehm occur, in which oblong-ellipsoid conidia are found with a *Dermatea* type of apothecium, but such species only serve to emphasize the close relationship between the two genera. The fact that the majority of *Pezicula* species do differ from the majority of *Dermatea* species in both the perfect and imperfect stages would seem to be sufficient justification for continuing to recognize *Pezicula* as a distinct genus.

The material on which this study is based was collected, for the most part, in the Temagami Forest Reserve, Ontario, and in the vicinity of Toronto, Ontario. Cultures were obtained from both ascospores and conidia and were grown on 2% malt extract agar and on sterilized twigs of the host. The twig cultures were prepared as described in an earlier paper, Groves (5). In only one of these species was the perfect stage obtained in culture, but the fact that the cultures from ascospores and conidia were similar and that both produced the same type of conidial fruiting body and spores in culture, was considered sufficient evidence of genetic connection.

Description of Species

Pezicula carpinea (Pers.) Tul. Sel. Fung. Carp. 3 : 183. 1865.

Peziza carpinea Pers. Syn. Fung. p. 673. 1801.
Cycledum carpinea Wallr. Flor. Crypt. Ger. 2 : 512. 1833.
Dermatea carpinea Fries. Summa veg. Scand. p. 362. 1849.
Octospora paradoxa Hedw. Obs. Bot. 1 : 13. 1802.
Ditiola paradoxa Fries. Syst. Myc. 2 : 171. 1822.
Ombrophila paradoxa Sacc. Syll. Fung. 8 : 620. 1889.
Pezicula fasciculata Dearn. and House. N.Y. St. Mus. Bull. 243 : 244. 1921.
Tubercularia fasciculata Tode. Fung. Meckl. tab. IV, fig. 3. 1790.
Cryptosporiopsis fasciculata Petrak. Ann. Myc. 21 : 186. 1923.
Discella discoidea Cke. and Pk. Ann. Rep. N.Y. St. Mus. 28 : 58. 1876.
Discula Peckiana Sacc. Syll. Fung. 3 : 675. 1884.
Peziza Betuli Alb. and Schw. Consp. Fung. p. 309. 1805.
Stictis Betuli Fries. Syst. Myc. 2 : 195. 1822.
Ocellaria Betuli Rehm. Rab. Kr. Fl. I, 3 : 136. 1889.
Tubercularíella Betuli v. Höhn. Z. Gärungsphysiol. 5 : 209. 1915.
?*Dermatella scotinus* Morg. J. Myc. 10 : 98. 1904.

Apothecia (Fig. 1 : 1) strongly erumpent, more or less in rows or sometimes scattered, caespitose, usually in elongated clusters, occasionally single, circular or distorted by crowding, narrowed below, substipitate, 0.5–1.0 mm. in diameter, 0.5–1.0 mm. in height, pale ochraceous yellow, slightly pruinose, brittle, rather waxy in consistency, more fleshy when moist; hymenium plane

to convex, slightly pruinose, ochraceous yellow to reddish orange, much brighter when moist, at first with a delicate lighter border which may disappear later; tissue of the hypothecium pseudoparenchymatous at the base, composed of irregular, hyaline cells, about 5–12 μ in diameter, often with host cells intermingled, becoming more prosenchymatous above, composed of more or less vertically parallel hyphae, 3–5 μ in diameter, in the upper part more interwoven and looser, often with intercellular spaces,

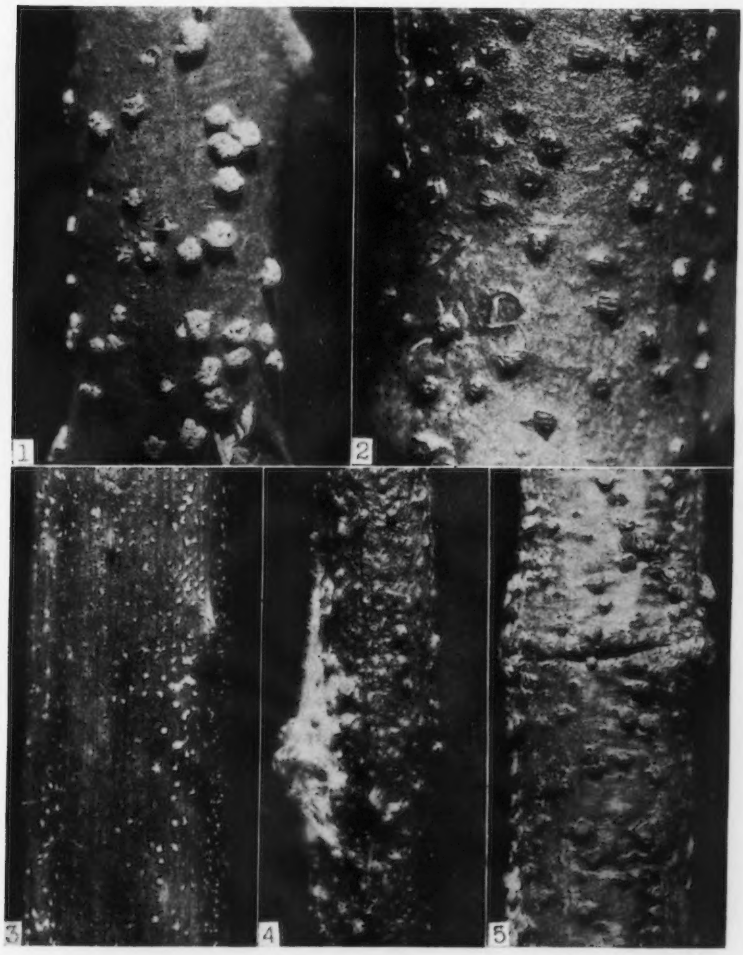

FIG. 1. *1. Apothecia of* Pezicula carpinea; *2. Conidial stage of* P. carpinea; *3. Conidial stage of* P. Rubi; *4. Apothecia of* P. Rubi *developed in culture; 5. Apothecia of* P. Corni. *All* ×4 *approx.*

toward the outside forming a pseudoparenchymatous zone, a few cells in thickness, of irregular, more or less isodiametric cells; subhymenium a narrow zone of slender, loosely interwoven hyphae; asci cylindric-clavate, usually tapering into a long stalk, eight-spored, sometimes four-spored, (120)–130–160–(190) × (14)–17–20–(22) μ; ascospores oblong-ellipsoid to ovoid, hyaline, straight or slightly curved, one- to four-celled, obliquely uniseriate or overlapping, (17)–20–30–(33) × (8)–10–12–(14) μ; paraphyses hyaline, filiform,

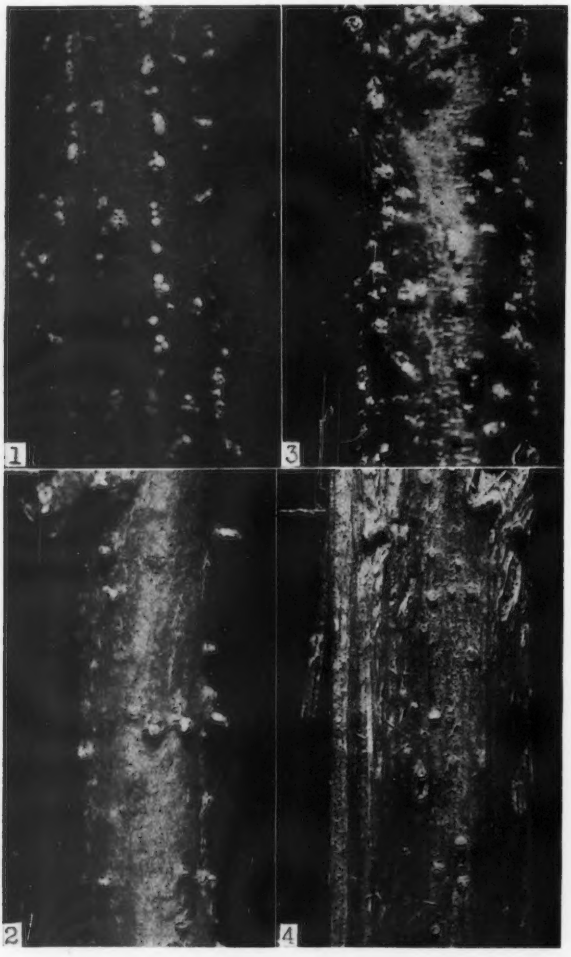

FIG. 2. *1. Apothecia of* Pezicula pruinosa; *2. Conidial stage of* P. pruinosa; *3. Conidial stage of* P. Corni *developed in culture; 4. Apothecia of* P. Rubi. *All* ×4 *approx.*

septate, simple or branched, 1.5–2.5 μ in diameter, the tips swollen, up to 6 μ in diameter and forming a slight epithecium. (Fig. 4 : 1b.)

Conidial fruiting bodies (Fig. 1 : 2) erumpent, more or less in rows or scattered over the twigs, cushion-shaped or wart-like, almost circular or elongated, 1–3 mm. in diameter, 0.3–1.0 mm. in height, dark reddish-brown, greyish, olivaceous, or almost black, hard, waxy, more fleshy when moist; tissue com-

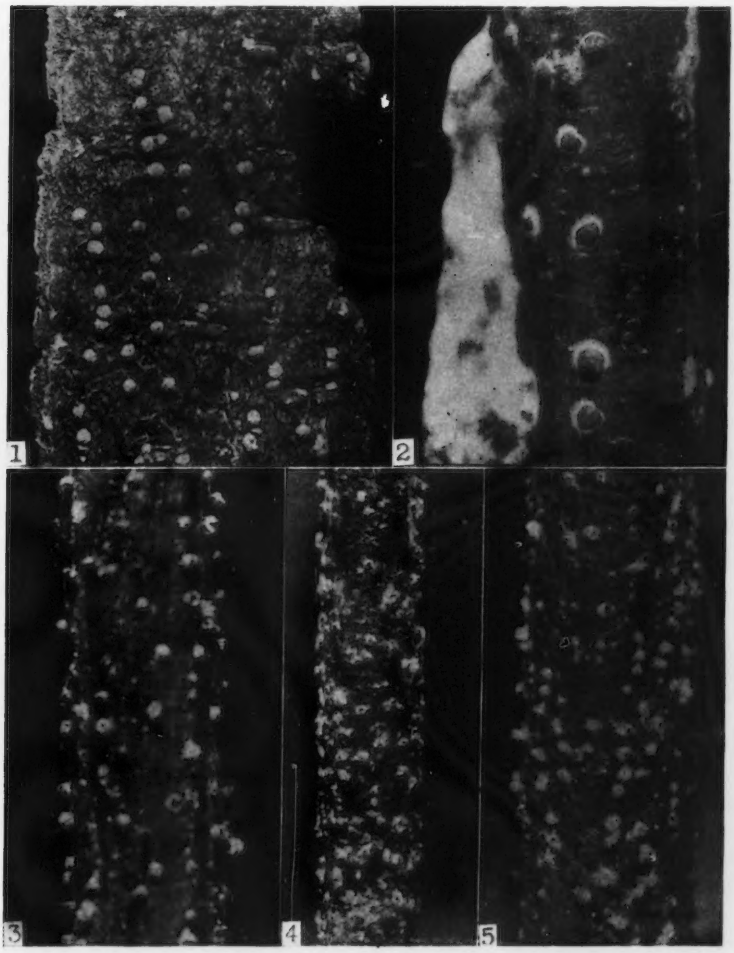

FIG. 3. *1. Apothecia of* Pezicula Hamamelidis; *2. Conidial stage of* P. carpinea *developed in culture; 3. Conidial stage of* P. pruinosa *developed in culture; 4. Conidial stage of* P. Rubi *developed in culture; 5. Conidial stage of* P. Hamamelidis *developed in culture. All* × *4 approx.*

posed of hyaline or pale yellowish, much branched, closely interwoven hyphae about 3–5 μ in diameter, in the upper central part more or less vertically parallel, spreading out obliquely toward the outside; conidiophores hyaline, septate, simple or branched, very variable in length, up to 100 μ, 2.5–3.0 μ in diameter and swollen up to 5–7 μ just below the tip; conidia oblong-ellipsoid, hyaline, straight or slightly curved, one end with a truncate apiculus, one-celled, 20–30–(34) $\times$ (10)–12–15 μ; microconidia hyaline, filiform, straight or curved, one-celled, 8–15 $\times$ 1.5–2.0 μ. (Fig. 4 : 1a.)

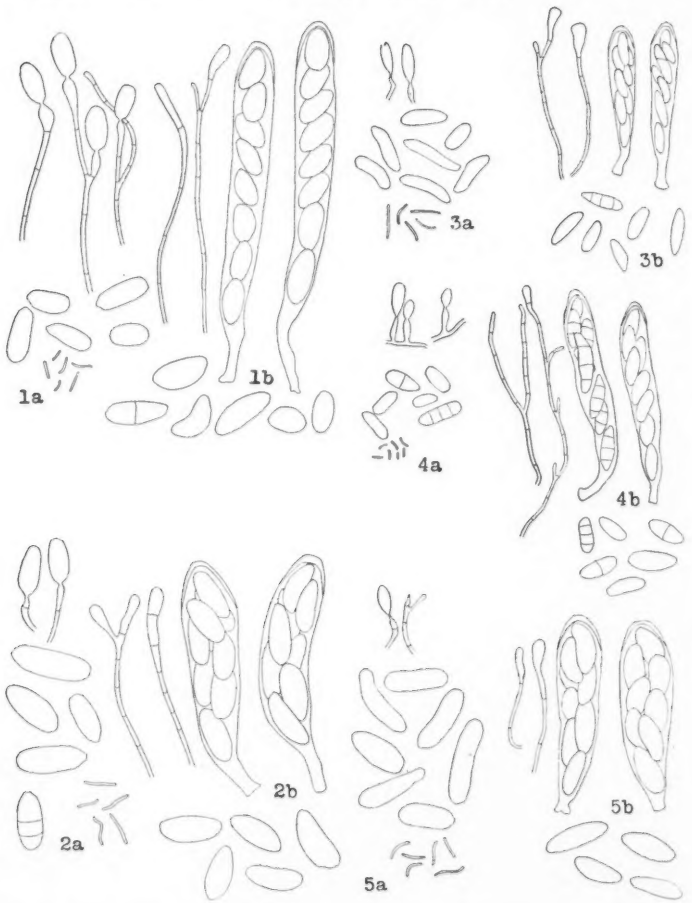

FIG. 4. *1.* Pezicula carpinea. *a. Conidia, conidiophores, and microconidia. b. Asci, ascospores, and paraphyses; 2.* P. Corni. *a. Conidia. conidiophores, and microconidia. b. Asci, ascospores, and paraphyses; 3.* P. Rubi. *a. Conidia. conidiophores, and microconidia. b. Asci, ascospores, and paraphyses; 4.* P. pruinosa. *a. Conidia, conidiophores, and microconidia. b. Asci, ascospores, and paraphyses; 5.* P. Hamamelidis. *a. Conidia, conidiophores, and microconidia. b. Asci, ascospores, and paraphyses. All* $\times$*400.*

HOST: *Carpinus caroliniana* Walt.

EXSICCATI: Ell. N. Am. Fung. *67b* (as *Dermatea carnea*), *2623*, *2741*, *3333*; Shear N. Y. Fung. *93*; Rel. Farl. *134*; Fung. Col. *921*; Wils. and Seav. Asc. and L.F. *91*; Krieg. Fung. Sax. *1029*; Weese Eum. sel. exs. *450*, *452*; Syd. Myc. Germ. *2729*.

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Carpinus caroliniana* Walt. *2905*, *3949 (65)**, *4377 (64)*, *7268 (287)*, *7394 (160)*, Toronto, Ontario—*6019 (157)*, Lynden, Ontario—*6570*, Brant Co., Ontario —ex U.S.D.A. Herb. F. P. *63042*, North Bloomfield, Conn.—ex Herb. Univ. Wis., Nekoosa, Wis., coll. J. J. Davis, June 8, 1918.

On *Carpinus* sp. Ex. Herb. Barbey-Boissier, Rel. Herb. Fuckel, Fung. rhen. *1129*.

Herbarium of J. W. Groves. On *Carpinus caroliniana* Walt. *58*, *59*, Toronto, Ontario—*570*, Constance Bay, Ontario—*264* Ithaca, N.Y.

On malt extract agar the colonies reach a diameter of about 3 cm. in three weeks. They are white, or cream coloured to buff, or slightly yellowish, with abundant, fluffy, aerial mycelium which is more or less tufted or in cushion-like heaps. They develop circular to elongated, rounded, fleshy stromata, up to 3 mm. in diameter and 2 mm. in height, which usually very soon become covered with white, downy mycelium and remain sterile, but occasionally produce conidia. In the few instances in which the production of conidia was observed, the conidiophores appeared to be enclosed at first in a more or less circular cavity in the stroma. This cavity soon split open widely at the top and the fruiting surface became convex and wholly exposed. The microscopic structure was similar to that found in nature, but the tissue is sometimes looser, especially at the outside. Both macro- and microconidia were produced. Sporulation seems to be rare, and when it does occur lasts only for a brief period, as the stroma quickly becomes overgrown with vegetative hyphae.

On twigs of *Carpinus*, aerial mycelium is usually very abundant, white, cream coloured, or buff, forming cottony cushions on the twigs or sometimes completely covering them. Sporulation is rare on the twigs also. The fruiting bodies (Fig. 3 : **2**) are erumpent, fleshy, rounded, or wart-like as in nature, 1 to 2 mm. in diameter and about 0.5 mm. in height, at first whitish, becoming reddish-brown and harder on drying. The conidiophores are borne over the outer surface and have not been observed to arise in a cavity. The microscopic features are similar to those found in nature. These fruiting bodies are also shortlived, and in a few days appear only as tufts of vegetative hyphae. It has been observed that if the conidial fruiting bodies as found in nature are put in a moist chamber, they very soon develop vegetatively in a similar manner.

Pezicula carpinea, as noted above, is considered to be the type species of the genus *Pezicula*. Its chief distinguishing characters are its occurrence on *Carpinus*, the yellow, waxy-fleshy apothecia in strongly erumpent clusters,

* *The numbers in brackets refer to duplicate collections in the writer's herbarium.*

the long asci, and the *Tubercularia*-like conidial stage. Brefeld (3) studied this species in culture, and his description of the cultures agrees well with the writer's cultures. He failed to obtain any fruiting bodies in culture, but this is not surprising as they are evidently formed only rarely, and then may easily be overlooked as the fruiting period is very brief.

This species is undoubtedly parasitic. Wagner (22) reported successful inoculations, and stated that in a 70- to 80-year-old stand of *Carpinus*, 140 out of 200 trees were killed by this fungus over a period of 10 years. On May 15, 1933, the writer inoculated two branches of a tree of *Carpinus caroliniana* in the following manner. A slit was cut in the bark with a flamed scalpel, and some agar and mycelium from a polysporous ascospore culture were inserted into the slit. The slit was then covered by a celluloid cylinder with the ends plugged with moist sphagnum, and the cylinder was removed after four days. A third branch was treated similarly but no inoculum was placed in the slit. On May 7, 1934, one of the inoculated branches had conidial fruiting bodies of *Pezicula carpinea* scattered along the branch for about 2 ft. on either side of the point of inoculation. The other inoculated branch and the check were healthy. Some of these conidial fruiting bodies were collected (Univ. of Tor. Herb. *7394*) and the fungus was isolated from the conidia. On Oct. 14, 1934, mature apothecia were thickly scattered along the branch for a distance of about 4 ft. from the point of inoculation, and the infection had evidently spread into the main trunk, as the entire upper part of the tree was dead. There were no fruiting bodies on the other inoculated branch or on the check, but both these branches were dead, having been involved in the death of the tree. The fungus was also isolated from ascospores (Univ. of Tor. Herb. *7268*).

Establishment of the genetic connection between *Pezicula carpinea* and *Cryptosporiopsis fasciculata* by cultural methods has merely confirmed a connection that has long been postulated on the basis of their close association. The conidial stage has been well described by Petrak (12) who observed a variation in which the stroma was less erumpent and evidently more like the conidial fruiting body of *Pezicula acericola* (Pk.) Sacc., described by Groves (6). The writer has not observed this condition in *P. carpinea*, but since this is the typical form of conidial stroma found in the genus, it would not be surprising to find this species sometimes taking this form. The few observations the writer was able to make of the formation of the conidial stroma on agar indicated that the spores were formed first in a cavity in the stroma as in other *Pezicula* species, but that the cavity soon spread open widely, wholly exposing the spore masses.

Some attempt has been made here to bring together the various synonyms of *P. carpinea*. The fungus known as *Ombrophila paradoxa* (Hedw.) Sacc. has been included on the strength of Tulasne's statement (21) that he considered it to be *P. carpinea*. Rehm (15) also mentioned it as a synonym. It would seem that *Peziza Betuli* Alb. and Schw. was based on the conidial stage of *P. carpinea*. The descriptions by Albertini and Schweinitz (1),

Fries (4), and Rehm (14) all omit mention of asci, and von Höhnel (7) recognized it as an imperfect fungus and referred it to the genus *Tubercülariella*. The writer has been unsuccessful in locating the type of *Dermatella scotinus* Morg., but there is nothing in the description to exclude it from *P. carpinea*.

Pezicula pruinosa Farlow, Mycologia 14 : 102. 1922.

Dermatea pruinosa Petrak, Ann. Myc. 20 : 196. 1922.

Sphaeronema pruinosum Peck, Ann. Rep. N.Y. St. Mus. 24 : 85. 1872.

Lagynodella pruinosa Petrak, Ann. Myc. 20 : 207. 1922.

Apothecia (Fig. 2 : 1) strongly erumpent, usually in long rows, sometimes single but usually in elongated clusters, circular, substipitate, 0.5–1.5 mm. in diameter, 0.5–1.0 mm. in height, pale yellow to orange yellow, darker toward the base, slightly pruinose above, becoming strongly greyish-pruinose toward the base, brittle, waxy in consistency, becoming more fleshy when moist; hymenium plane to slightly convex, pruinose when dry, margin paler, slightly raised at first, later disappearing; tissue of the hypothecium compact, pseudoparenchymatous, often with host cells intermingled, composed of hyaline cells about 5–12 μ in diameter, sometimes more elongated up to 20 μ, arranged in more or less oblique rows toward the outside and thicker walled, and with a granular encrustation on the outside of the apothecium; subhymenium a zone of rather loosely interwoven, slightly coloured hyphae; asci cylindric-clavate, short-stalked, eight-spored, (75)–90–110–(130) × (12)-14-17–(20) μ; ascospores oblong-ellipsoid to ovoid, hyaline, sometimes slightly coloured, one- to four-celled, occasionally muriform, straight or slightly curved, irregularly biseriate, (14)–17–25–(30) × (6)–7–9–(10) μ; paraphyses hyaline, filiform, septate, branched, 2.0–2.5 μ in diameter, swollen at the tip up to 5 μ and forming a slight epithecium. (Fig. 4 : 4b.)

Conidial fruiting bodies (Fig. 2 : 2) erumpent, scattered or more or less in rows, usually separate, sometimes with two or three arising from the same basal stroma, cylindric-conic, 0.5–2.0 mm. in height, 0.2–0.4 mm. in diameter at the base and tapering to 150–300 μ at the tip, greyish-pruinose, hard, horny in consistency, becoming more fleshy when moist, the conidia emerging through an irregular opening at the tip or side of the tip of the beak; tissue of the basal stroma composed of thick-walled cells, in the central part almost isodiametric, 5–10 μ in diameter, toward the outside more elongated, 12–20 × 5–8 μ, and curving upward in parallel rows, the walls of the beak about 50–75 μ in diameter, consisting of two zones, an outer zone of dark, thick-walled, almost isodiametric cells, and an inner zone of lighter coloured, more elongated cells in parallel rows; conidiophores hyaline, continuous or septate, sometimes branched, 12–30 × 2.0–2.5 μ, swollen below the tip up to 5 μ, lining the beak and the elongated to ovoid cavity in the basal stroma; conidia oblong-ellipsoid, hyaline, one-celled, straight or slightly curved, ends rounded; one end with a truncate apiculus, 15–25 × 7–10 μ; microconidia have not been observed. (Fig. 4 : 4a.)

HOST: *Amelanchier* species.

TYPE: Rel. Farl. *135b.*

EXSICCATI: Rel. Farl. *135a, b;* Fung. Dak. *450.*

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Amelanchier canadensis* (L.) Medic. *3407 (14), 3507, 4513, 7987 (319), 7988 (394),* Temagami Forest Reserve, Ontario—ex herb. D. H. Linder, Wellesley, Mass., coll. D. H. Linder, June 5, 1935—ex Cornell Univ. Pl. Path. Herb. *24741,* Lloyd Preserve, McLean, N.Y.

Herbarium of J. W. Groves. On *Amelanchier intermedia* Spach. *263,* McLean Swamp, Ithaca, N.Y., comm. G. E. Thompson.

On *Amelanchier* sp. *438,* Kingsmere, Quebec—*616,* Duchesnay, Quebec.

On malt extract agar the colonies reach a diameter of 3.5–4.5 cm. in two weeks. The mycelium is at first almost colourless, closely appressed to the agar, and somewhat sodden; later whitish to pale grey-green, aerial mycelium develops in unevenly distributed tufts. The conidial fruiting bodies develop as rounded or conical, fleshy stromata, up to 2 mm. in diameter and 3 mm. in height, pale greenish to brownish, and usually covered with white, downy to cottony mycelium, and containing one or more rounded or irregularly lobed cavities, tearing open irregularly at the top or sides. They are composed of interwoven, hyaline or slightly yellowish hyphae, 2–4 μ in diameter, the cavities surrounded by a compact, narrow zone about 25–50 μ in thickness, and sometimes darker coloured. The conidiophores may be longer and more branched than in nature. The conidia are typical. Microconidia are usually abundant, hyaline, filiform, straight or curved, one-celled, 6–10 $\times$ 1.5–2.0 μ.

On twigs of *Amelanchier* there is usually more or less abundant, white, cottony, aerial mycelium which forms tufts around the point of inoculation or may spread over the twig. The conidial fruiting bodies (Fig. 3 : 3) develop better when there is less aerial mycelium. They are erumpent, more or less in rows, usually irregularly rounded, up to 1 mm. in diameter and about the same in height, splitting open irregularly at the top or side, but sometimes developing a cylindric-conic ostiole which is usually shorter than in nature, fleshy, drying hard and horny, greyish-pruinose, black or greenish at the tip. The tissue structure is similar to that found in nature, and the conidiophores and conidia are typical.

Establishment by cultural methods of the genetic connection between *Pezicula pruinosa* and *Sphaeronema pruinosum* is also merely the confirmation of a previously suggested relationship. This connection was suggested by Farlow in his original description published by Thaxter (19), and also by Sydow and Petrak (18). The conidial stage was collected in the Temagami region in the spring of 1932 when this study was commenced, but although search was made for it, the perfect stage was not found until 1935. In June, 1934, a tree with abundant conidial fructifications was selected and kept under observation until the middle of September, but no apothecia were produced. The

following year it was again kept under observation, and on July 2, 1935, mature apothecia were found.

The chief distinguishing characters of this species are its occurrence on *Amelanchier*, the yellow, waxy-fleshy, substipitate apothecia which are markedly greyish-pruinose near the base, and the *Sphaeronema*-like conidial stage. The conidial stage was first described by Peck (10) as a *Sphaeronema*, and Petrak (18) made it the type of his new genus *Lagynodella*. It is doubtful if *Sphaeronema pruinosum* Berk. and Curt. described on *Rhus venenata* is the same fungus.

Pezicula Corni Petrak, Ann. Myc. 20 : 197. 1922.

Dermatea Corni Phill. and Hark., Grev. 13 : 22. 1884.

Ocellaria aurea var. *cornicola* Kauffm. and Kanouse, Papers Mich. Acad. Sci., Arts, and Letters, 9 : 183. 1929.

Sphaeropsis cornina Peck, Ann. Rep. N.Y. St. Mus. 32 : 38. 1879.

Macrophoma cornina Sacc., Syll. Fung. 10 : 192. 1892.

Cryptosporiopsis cornina Petr. and Syd., Ann. Myc. 22 : 370. 1924.

Apothecia (Fig. 1 : 5) erumpent, sub-immersed when dry, becoming more strongly exserted when moist, scattered or more or less in rows, separate, sometimes in small clusters, circular, sessile, slightly narrowed below, 0.4–1.0 mm. in diameter, 0.2–0.4 mm. in height, ochraceous yellow to dull reddish-orange, slightly pruinose above, becoming darker to almost black below, soft, waxy, crumbling easily, more fleshy when moist; hymenium plane to convex, slightly pruinose, with a slight margin; tissue of the hypothecium compact, pseudoparenchymatous, composed of slightly coloured, irregular, angular cells about 5–15 μ in diameter, arranged in more or less vertically parallel rows curving obliquely toward the outside, golden yellow in the central part, becoming much darker toward the outside, cells more elongated in the upper part; subhymenium a zone of closely interwoven hyphae; asci broadly clavate or subellipsoid, short-stalked, eight-spored, (85)–105–130–(145) × (20)–23–30–(33) μ; ascospores oblong-ellipsoid to ovoid, hyaline or slightly yellowish, one- to four-celled, straight or slightly curved, irregularly biseriate, (22)–25–35–(40) × (10)–12–15–(17.5) μ; paraphyses hyaline, filiform, septate, simple or branched, 2.0–3.0 μ in diameter, the tips often coloured and swollen up to 5 μ, forming a slight epithecium. (Fig. 4 : 2b.)

Conidial fruiting bodies erumpent, scattered, mostly separate, occasionally two or three together, black, circular, rounded to conical, opening widely and irregularly, 200–350 μ in diameter, 300–450 μ in height, containing a single, slightly lobed cavity; the basal stroma a pseudoparenchymatous layer 25–35 μ in thickness, composed of dark, thick-walled cells, 5–8 μ in diameter, in the central part the stroma usually much thicker, up to 100 μ, and the tissue composed of lighter coloured, closely interwoven hyphae; conidiophores hyaline, continuous or sometimes septate, simple or branched, cylindric or conical, sometimes swollen slightly below the tip, 10–30 × 3–6 μ; conidia

oblong-ellipsoid to ovoid, hyaline or slightly yellowish, straight or slightly curved, one- to four-celled, ends rounded, one end with a truncate apiculus, (25)–30–35–(40) × (9)–11–13–(15) μ; microconidia hyaline, filiform, curved or sometimes almost straight, one-celled, 12–18 × 1.5–2.0 μ. (Fig. 4 : 2a.)

HOST: *Cornus* species.

TYPE: On *Cornus stolonifera* Michx. Priest River, Bonner Co., Idaho, J. R. Weir, *16909*. Oct. 11, 1918.

EXSICCATI: Ell. N. Am. Fung. *2809* (as *Pezicula rhabarbarina*).

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Cornus stolonifera* Michx. *4833 (138)*, Toronto, Ontario—ex U.S. D. A. Path. and Myc. Coll. Herb. J. R. Weir *16909*, part of type—ex Farl. Herb. Harvard Univ., coll. G. D. Darker *(5484)*, Waltham, Mass.

On *Cornus circinata* L'Her. *4836 (126)*, *4839 (129)*, Ottawa, Ontario—*6564 (280)*, Aurora, Ontario—*5699*, *6590*, *(243)*, *7974*, *7975 (449)*, *7976*, Temagami Forest Reserve, Ontario.

On *Cornus alternifolia* L. Ex Univ. of Mich. Crypt. Herb. Part of type of *Ocellaria aurea* var. *cornicola* Kauffm. and Kanouse, Rock River, Mich. Sept. 4, 1927.

On *Cornus* sp. *6940*, Aurora, Ontario—*7977*, Temagami Forest Reserve, Ontario—ex herb. L. O. Overholts *13370*, Hawn's Bridge, Hunt. Co., Pa.

Herbarium of J. W. Groves. On *Cornus* sp. *553*, *554*, Casey's Corners, Nova Scotia—*584*, Ottawa, Ontario.

Harkness No. *2259*, the type of *Dermatea Corni* Phill. and Hark. A single apothecium in the Durand Herbarium of Discomycetes, Cornell University *3786*, and a slide from the specimen in Kew Herbarium.

On malt extract agar the colonies reach a diameter of 2–3 cm. in three weeks. They are whitish to greenish-grey, sometimes slightly yellowish to orange at the centre, the aerial mycelium loose and fluffy, then becoming slightly matted. The conidial fruiting bodies are almost globose to subcylindric, dark greenish to almost black, fleshy, glabrous or covered with a short, grey mycelium, about 150–350 μ in diameter, sometimes larger, tearing open widely and irregularly at the top, and containing a single, rounded or somewhat lobed cavity; tissue composed of closely interwoven, brownish hyphae, 2–5 μ in diameter; conidiophores, conidia, and microconidia as found in nature.

On twigs of *Cornus* there is usually very little aerial mycelium, but sometimes greenish or brownish tufts appear at the point of inoculation. The twigs become thickly covered with small, black, erumpent stromata, more or less in rows, at first rounded and covered with short, reddish-brown, hair-like hyphae, then sometimes elongating and becoming cylindric-conic, opening widely at the top. These fruiting bodies (Fig. 2 : 3) are very similar in structure to the fruiting bodies as found in nature, and produce typical conidia and microconidia.

The chief distinguishing characters of this species are its occurrence on *Cornus*, the ochraceous-orange, waxy apothecia, not strongly erumpent and usually single, the broad asci, large spores, and the *Macrophoma*-like conidial stage. As far as is known, the genetic connection of *Pezicula Corni* and *Cryptosporiopsis cornina* has not been reported previously. The *Myxosporium* species reported by Seaver (17) as the conidial stage of this species is undoubtedly *C. cornina*. This species has been confused to some extent with *Pezicula Rubi* (Lib.) Niessl, from which it can readily be distinguished by the size of the asci and spores and the type of conidial stage. Probably all the records of *P. Rubi* on *Cornus* will prove to be *P. Corni*.

Seaver (17) has drawn attention to the fact that the identity of *Dermatea Corni* Phill. and Hark., an earlier name, is uncertain. The writer has made some effort to locate the type of this species, which should be Harkness' No. *2259* according to the original description. In the Durand Herbarium of Discomycetes at Cornell University No. *3786*, is a packet labelled *Dermatea Corni* Phill. and Hark. Co-type, and bearing the above Harkness number. This packet contains a single apothecium detached from the substrate, but which is unquestionably a *Pezicula*, and is similar to *P. Corni* Petr. in gross appearance. Some of the writer's material was sent to Mr. E. W. Mason of the Imperial Mycological Institute, with the request that he compare it with the type material of *Dermatea Corni* if any were to be found in the Phillips Collection. Mr. Mason kindly did this and reported that the specimen in the Phillips Collection was very scanty and seemed mostly immature, but was evidently the same fungus. Examination of a slide which Mr. Mason loaned to the writer showed that although the fungus was immature, some of the characteristic large asci and spores were present.

The writer is, therefore, convinced that *Dermatea Corni* Phill. and Hark. is identical with *Pezicula Corni* Petr. The fungus is a *Pezicula*, but since Petrak has used the combination *P. Corni*, it would now be necessary to give the fungus a new name in order to conform strictly to the rules. However, in view of the fact that the type material of *Dermatea Corni* is scanty and immature, and that Petrak's name is established in the literature and is eminently suitable, it would seem preferable to retain this name.

Pezicula Rubi (Lib.) Niessl, Rabh. fung. Eur. n. 2122. 1876.

Patellaria Rubi Libert, Pl. Crypt. Ard. n. 231. 1834.

Helotium Rubi Spree, Rabh. fung. Eur. n. 717. 1865.

Dermatea Rubi Rehm, Rabh. Krypt. Fl. I, 3 : 258. 1889.

Peziza arduennsis Montg., Ann Sci. Nat. II, T. V. p. 287, t. 13, f. 5. 1836.

Gloeosporium phaeosorum Sacc., Mich. I : 360. 1878.

Myxosporium phaeosorum Allescher, Rabh. Krypt. Fl. I, 7 : 529. 1902.

Discosporium phaeosorum v. Höhn., Z. Gärungsphysiol. 5 : 197. 1915.

Discosporiella phaeosora Petrak, Ann. Myc. 21 : 14. 1923.

Apothecia (Fig. 2 : 4) erumpent, scattered, separate or sometimes two or three in a cluster, circular, sessile, narrowed below, 0.2–1.0 mm. in diameter, 0.2–0.5 mm. in height, dull ochraceous yellowish to reddish orange, brighter when moist, slightly pruinose, brittle, waxy in consistency, more fleshy when moist; hymenium at first concave, then plane to convex, slightly pruinose, margin at first raised, later disappearing; tissue of the hypothecium pseudoparenchymatous, at the base composed of hyaline to yellowish-brown, thick-walled cells, 3–8 μ in diameter, more elongated above and somewhat interwoven, toward the outside in obliquely parallel rows, dark, and thick-walled, 5–12 μ in diameter, sometimes the outer cells projecting slightly and clavate; subhymenium a narrow zone of closely interwoven hyphae; asci cylindric-clavate, more or less stalked, eight-spored, (75)–85–100–(115) × (10)–12–15–(17) μ; ascospores oblong-ellipsoid to ovoid or subfusiform, hyaline, straight or slightly curved, one- to four-celled, irregularly biseriate, (15)–18–25–(30) × 5–8 μ; paraphyses hyaline, filiform, septate, simple or branched, 1.5–2.0 μ in diameter, swollen at the tips up to 5–6 μ, or sometimes only slightly swollen, and forming a slight epithecium. (Fig. 4; 3b.)

Conidial fruiting bodies (Fig. 1 : 3) thickly scattered, developing below or within the epidermis and splitting it, forming little blisters but scarcely breaking through, 150–300 μ in diameter, circular or elliptical, consisting of a thin basal layer 5–8 μ in thickness, composed of hyaline, indistinct hyphae from which the conidiophores arise; conidiophores hyaline, continuous or septate, not observed branching, conical or cylindric, sometimes slightly swollen below the tip, 5–12 × 1.5–2.0 μ; conidia oblong-ellipsoid to elongated, hyaline or pale yellowish-green, one-celled, straight or slightly curved, ends rounded or slightly tapering, one end with a truncate apiculus, (15)–20–28–(33) × (5)–6–7.5–(8.5) μ; no microconidia observed. (Fig. 4 : 3a.)

HOST: *Rubus* species.

TYPE: Libert Pl. Crypt. Ard. Fasc. III, *231.*

EXSICCATI: Rel. Farl. *114*; Fung. Col. *1120* (as *Pezicula rhabarbarina*); Kr. Fung. Sax. *41*, *1146.*

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Rubus* species. *6813 (270)*, *6843 (283)*, Toronto, Ontario—*6960 (276)*, Bell's Lake, north of Parry Sound, Ontario—*7403 (292)*, Mt. Nemo, north of Nelson, Ontario—*7990 (419)*, Temagami Forest Reserve, Ontario.

Herbarium of J. W. Groves. On *Rubus* species. *505*, Graham Bay, Ontario—*622*, Constance Bay, Ontario.

Durand Herbarium of Discomycetes. *3666*, ex Lib. Pl. Crypt. Ard. *231.* part of type.

On malt extract agar the colonies reach a diameter of 4.5–5.0 cm. in two weeks. The mycelium is sodden and closely appressed, with scant, fluffy to matted, more or less tufted, whitish to slightly pinkish, aerial mycelium. The conidial fruiting bodies are rounded, almost globose, black or greenish-black, usually covered with a white, downy mycelium, 0.2–3 mm. in diameter,

containing one or sometimes several, rounded or lobed cavities, tearing open widely and irregularly. The tissue is composed of hyaline, closely interwoven hyphae, about 2–3 μ in diameter, looser toward the outside. The cavity is surrounded by a zone of compact, pseudoparenchymatous tissue, composed of small, irregular cells, 3–7 μ in diameter. The conidiophores and conidia are typical. Microconidia present, hyaline, filiform, one-celled, straight or curved, 10–30 $\times$ 1.5–2.0 μ.

On twigs of *Rubus* the aerial mycelium is usually not abundant, forming a white or buff, cottony tuft at the point of inoculation, and occasionally spreading over the twig. The conidial fruiting bodies (Fig. 3 : 4) are erumpent, breaking through the epidermis and developing chiefly on the surface of the twig, rounded to cylindric, 0.3–1.0 mm. in diameter, 0.2–0.6 mm. in height, brownish to blackish, usually covered with a loose, whitish to brownish mycelium. The basal stroma may remain thin as in nature, or may develop to a thickness of 250 μ and contain one or more cavities in the upper part. The tissue is composed of closely interwoven hyphae, looser at the outside, sometimes pseudoparenchymatous at the base. The conidia and conidiophores are typical.

In one twig culture originating from mass ascospores, several apothecia were produced (Fig. 1 : 4). They were typical of the apothecia as found in nature in both macroscopic and microscopic characters, and cultures from the ascospores were typical.

Some confusion has existed regarding the specific identity of this fungus, and apparently the name has been applied to more than one species. Evidently the type may be considered to be the specimen issued in Pl. Crypt. Ard. Fasc. III, No. *231*, and a small fragment of this specimen in the Durand Herbarium of Discomycetes at Cornell University No. *3666*, was examined. This packet bore the label "*Patellaria Rubi* Lib. on fallen branches of *Rubus*" and contained only two apothecia detached from the substrate, but they were evidently the same fungus that occurs on *Rubus* in this region, and the determination of the writer's material is based on this specimen and also that issued in Kr. Fung. Sax. *41*.

The chief distinguishing characters of this species are its occurrence on *Rubus*, the ochraceous, waxy, mostly single apothecia, narrow asci and spores, and the *Gloeosporium*-like conidial stage. The genetic connection of *Pezicula Rubi* and *Discosporiella phaeosora* was suggested by Petrak (11), and this has been confirmed by the cultural studies above. Petrak (11) based the genus *Discosporiella* on this species, characterizing it as a *Discosporiopsis* (= *Cryptosporiopsis* Bub. & Kab.) with a very thin basal stroma. Nannfeldt (8) took the view that this was not a satisfactory character on which to base a generic separation, and in the writer's cultures the basal stroma has varied from 10–250 μ in thickness, thus supporting Nannfeldt's contention.

The writer is of the opinion that *Pezicula Rubi* occurs only on *Rubus*, although it has been reported on other hosts. The type of *P. Rubi* was on *Rubus* as noted above. Berkeley (2) described a fungus as *Peziza rhabarbarina*,

which he said occurred on wild rose, and which Tulasne (21) later transferred to *Pezicula*. Saccardo (16) also described a *Pezicula* occurring on rose as *Pezicula Rosae*. Phillips (13) considered *P. Rosae* to be a synonym of *P. rhabarbarina* and *P. Rubi* to be distinct; but Rehm (14) considered *P. rhabarbarina* to be a synonym of *P. Rubi* and *P. Rosae* to be distinct. Rehm gave the host of *P. Rubi* as "an dürren Ranken von *Rubus fruticosus*, seltener von wilder *Rosa*", and of *P. Rosae* as "an dürren Aesten von *Rosa canina*". In addition *P. Rubi* has been reported on *Cornus* and *Hamamelis*. The cultural and morphological studies reported in this paper have demonstrated that there are three distinct species occurring on *Rubus*, *Cornus* and *Hamamelis*, and that these three species can be separated in gross appearance, microscopic characters, cultural characters, and especially by the type of conidial stage. Examination of all available collections and exsiccati has, so far, confirmed this separation on the basis of hosts. The question as to whether *P. Rubi* may occur also on rose must remain undecided for the present. The writer has had no opportunity of examining fresh material of the fungus on rose, and its conidial stage is unknown, but examination of the specimen in Brenckle Fung. Dak. *392*, labelled *Pezicula Rosae* Sacc., shows that it is evidently distinct from all three of the above species. The writer is of the opinion, therefore, that Phillips' interpretation is more probably the correct one and that these fungi are more closely restricted to the host than has been considered heretofore. It is not inconceivable that *P. Rubi* might occasionally occur on rose, but since there evidently is a distinct species occurring on rose, reports of the occurrence of *P. Rubi* on this host should be supported by cultural evidence showing the type of conidial stage.

Nannfeldt (9) concluded from an examination of the type specimen of *Helotium scoparium* Cke. that it was only *Pezicula Rubi* in a bad state of preservation. The writer would also question this determination, on the grounds of host relationship. *H. scoparium* was described on *Sarothamnus* of the Leguminosae, and the occurrence of *P. Rubi* on this host would seem most unlikely.

Pezicula Hamamelidis Groves and Seaver sp. nov.

Apotheciis erumpentibus, dispersis vel plus minus seriatim instructis, solitariis vel caespitosis, sessilibus, versus basim attenuatis, orbicularibus, 0.3–1.0 mm. latis, 0.2–0.4 mm. altis, ochraceo-flavis, mollibus, in sicco ceraceis, in humido carnosis; hymenio plano vel convexo, leviter pruinoso, margine primo pallido dein evanescente; hypothecio pseudoparenchymato; ascis oblongo-clavatis, brevissimo stipitatis, octosporis, 90–120 × 20–25–(30) μ; ascosporis elliptico-oblongis vel ovoideis, hyalinis, rectis vel leviter curvatis, continuis vel triseptatis, (20)–25–33–(37.5) × (8)–9–11–(13) μ; paraphysibus hyalinis, filiformibus, septatis, simplicibus vel ramosis, 2–3 μ diam., apice ad 8 μ incrassatis, leve epithecium formantibus.

Apothecia (Fig. 3 : 1) erumpent, scattered or more or less in rows, usually single, sometimes caespitose with two to six in a cluster, sessile, narrowed below, circular, 0.3–1.0 mm. in diameter, 0.2–0.4 mm. in height, pale ochraceous yellowish, close to cinnamon buff when dry, brighter, close to

warm buff when moist, brittle, waxy in consistency, more fleshy when moist; hymenium plane or convex, slightly pruinose, at first with a delicate, pale border which later disappears; tissue of the hypothecium compact, pseudoparenchymatous, composed of thick-walled, more or less isodiametric to somewhat elongated cells about 5–10 μ in diameter, hyaline to golden yellow, arranged in more or less vertically parallel rows which curve obliquely toward the outside where they are darker coloured; subhymenium narrow, composed of rather loosely interwoven hyphae about 3–5 μ in diameter; asci oblong-clavate, broad, short-stalked, eight-spored, 90–120 × 20–25–(30) μ; ascospores oblong-ellipsoid to ovoid, hyaline, straight or slightly curved, one- to four-celled, irregularly biseriate, (20)–25–33–(37.5) × (8)–9–11–(13) μ; paraphyses hyaline, filiform, septate, simple or occasionally branched, 2–3 μ in diameter, the tips clavate and swollen up to 8 μ in diameter, forming a slight, yellowish epithecium. (Fig. 4 : 5b.)

Conidial fruiting bodies minute, about 300–450 μ in diameter, developing beneath the outer bark layers and splitting them slightly, the conidia emerging in whitish masses. The fruiting body consists of a cushion-shaped basal stroma, usually much thicker in the centre than at the periphery, and containing one or possibly more cavities; tissue of the basal stroma pseudoparenchymatous, composed of hyaline, irregular cells about 3–8 μ in diameter, becoming more elongated and interwoven in the thicker central part, the cavities surrounded by a narrow zone of hyaline, slender, indistinct hyphae; conidiophores hyaline, cylindric, septate, simple or sometimes branched, 15–30 × 2.5–3 μ, swollen below the tip up to 4 μ; conidia oblong-ellipsoid, hyaline, one-celled, straight or slightly curved, one end with a truncate apiculus, (25)–30–37–(45) × (9)–12–14.5 μ; microconidia borne in the same fruiting body, hyaline, filiform, straight or curved, one-celled, 10–18 × 1.5–2.0 μ. (Fig. 4 : 5a.)

HOST: *Hamamelis virginiana* L.

TYPE: University of Toronto Herbarium *7983*, Don Valley, Toronto, Ontario. Coll. J. W. Groves, Oct. 26, 1935.

EXSICCATI: Syd. Fung. exot. exs. *423* (as *Dermatea Rubi*).

SPECIMENS EXAMINED: University of Toronto Herbarium. On *Hamamelis virginiana* L. *2755, 4373 (54), 4375 (55), 4380 (59), 7982, 7983 (443).* Toronto, Ontario—*7984*, Brant Co., Ontario.

Herbarium of J. W. Groves. On *Hamamelis virginiana*. *56, 272*, Toronto, Ontario.

On malt extract agar the colonies reach a diameter of about 4 cm. in two weeks, producing a white to pale yellowish or brownish, loosely matted, to fluffy or cottony aerial mycelium. Conidial stromata are formed only occasionally and are usually much larger than in nature, up to 2 mm. in diameter and 1 mm. in height, more or less rounded, fleshy, buff, brownish, or olivaceous, becoming covered with a white, downy mycelium, at first with drops of clear green or amber liquid on them, then splitting open widely and irregularly,

exposing the whitish spore masses; the tissue composed of hyaline, rather loosely interwoven hyphae, 2.5–4.0 μ in diameter, with a narrow, compact zone of closely interwoven hyphae surrounding the cavity. The conidia and conidiophores are typical and microconidia are very abundant.

On twigs of *Hamamelis* a thin, white, cottony, aerial mycelium is sometimes formed but is usually not abundant. The conidial fruiting bodies (Fig. 3 : 5) are not formed abundantly and sporulation is rare. They are usually strongly erumpent, rounded or irregular in shape, sometimes subcylindric, covered with a white, downy mycelium. The tissue of the stroma is pseudoparenchymatous at the base, composed of hyaline, irregular cells, 5–15 μ in diameter, becoming more prosenchymatous in the upper part, the hyphae rather loosely interwoven but with a compact, yellowish zone, 20–40 μ in thickness, surrounding the cavity. Microconidia are usually abundant while macroconidia are only occasionally produced.

This species was collected near Toronto by Professor H. S. Jackson, and sent to Dr. F. J. Seaver before the writer had begun the study of this group. Dr. Seaver recognized it as an undescribed species, proposing the manuscript name *Pezicula Hamamelidis*, and subsequently suggested, when this cultural study was made, that the writer describe the species.

The chief distinguishing characters of this fungus are its occurrence on *Hamamelis*, the ochraceous, waxy, mostly single and not strongly erumpent apothecia, broad asci, and the *Cryptosporiopsis* type of conidial stage. The asci are usually shorter and the ascospores narrower than in *Pezicula Corni*, and both asci and ascospores are broader than in *P. Rubi*. It has evidently been confused with the latter in the past, but it can readily be distinguished by the size of the asci and ascospores as well as by the size of conidia and type of conidial fruiting body. Cultures of the two forms are quite different in appearance also, and there is no doubt that they are distinct species.

The conidial fruiting bodies are extremely inconspicuous, and were only discovered by placing twigs bearing young apothecia in a moist chamber and observing the masses of conidia which emerged through tiny cracks in the bark. As far as could be ascertained the conidial stage is also undescribed, but it is a typical *Cryptosporiopsis*, and the majority of *Pezicula* species which the writer has studied have had conidial stages of essentially the same form as that found in *P. Hamamelidis*.

Conclusion

In these five species, all belonging to the genus *Pezicula*, we thus find conidial stages that could be referred to widely separated families of the Fungi Imperfecti; for example, the conidial stage of *P. carpinea* could be referred to the Tuberculariaceae, of *P. pruinosa* to the Phomaceae, and of *P. Rubi* to the Melanconiaceae. In culture, although retaining some of the characters as found in nature, these conidial stages nevertheless tend to approach a common form, which consists of a more or less globose stroma containing one or more cavities. This is probably the primitive type of stroma in this genus, from

which the other types have evolved. It is of interest to note that in spite of the range of variation in the conidial stroma, the conidial spore itself remains rather constantly oblong-ellipsoid in form. The form of the spore in this group may, therefore, be considered of greater importance in indicating relationships than the form of the stroma. This will be more fully discussed after species of the related genus *Dermatea* have been considered.

Acknowledgments

The writer wishes to express his thanks to Professor H. S. Jackson, under whose direction the work was carried on, for his continued interest and helpful suggestions; to Professor H. M. Fitzpatrick for his kindness in making available for examination the specimens in the Durand Herbarium of Discomycetes; and to Mr. E. W. Mason for examining the type specimen of *Dermatea Corni* Phill. and Hark. and comparing it with the writer's material.

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BULB NEMATODE CONTROL IN IRIS BY HOT WATER[1]

By R. J. Hastings[2], J. E. Bosher[3] and William Newton[4]

Abstract

The immersion of iris bulbs in water at 110 to 112° F. for 60 min. effectively destroys the bulb nematode.

The safe period for nematode destruction in iris by hot water lies between July 26 and August 9. Thereafter injury to the bulbs occurs and is progressively greater as the immersion dates are delayed.

Introduction

The hot water treatment is an efficient method for the control of the bulb nematode in narcissi when the bulbs are immersed for three hours at 110 to 112° F. shortly after they are lifted.

A three-hour immersion period was found to be highly injurious to iris bulbs, hence a study was undertaken to ascertain whether a shorter immersion period might destroy the bulb nematode in iris without injuring significantly the vitality of the bulbs. At the outset, there appeared to be little hope that a shorter immersion period would prove effective, for there is little evidence that a distinct strain is involved in iris parasitism (4). Sherman (5) and Hastings and Newton (3) have reported bulb nematode survivals in narcissus after an immersion for three hours at 110 to 112° F. These survivals were usually pre-adults. In narcissus the pre-adult stage predominates in the nematode populations after long storage periods. Hastings and Bosher (2) have analyzed the nematode populations in a few varieties of iris and have reported small populations that consist largely of young larvae. Also, where large populations were found, the analyses revealed that they were principally young larvae. As our studies of the heat resistance of mixed stage cultures (3) showed that young larvae are destroyed within 60 min. at 110 to 112° F., it seemed probable that a 60-min. immersion period would prove effective in the control of the bulb nematode in iris, providing the bulbs themselves were not significantly injured by the heat treatment.

The English and American experiments covering the effect of hot water immersion upon iris have been briefly reviewed by Goodey (1) in his presidential address to the Association of Applied Biologists in 1936. He stated that the attempts of Steiner and Buhrer (Washington, D.C.) and Hodson (England) to control the bulb nematode in iris by hot water were not successful. On the other hand, he stated that Staniland successfully controlled the

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nematode in "Imperator" bulbs by immersing them in early autumn for 50 min. at 110 to 112° F.

In correspondence, Dr. F. Weiss reports that in 1936 a quantity of North Carolina iris bulbs were put through a one- to three-hour immersion in water at 110 to 112° F. without injury, between July and the end of August, but severe injury occurred when iris bulbs were similarly treated in mid-October. Dr. Weiss states that the bulbs grown in the southeastern United States attain a greater degree of dormancy than those grown in the northwest. It is probable therefore that the safe immersion period in British Columbia may be shorter than in North Carolina.

Experimental

Parcels of "Supreme", "Imperator", and "Wedgewood" iris bulbs were immersed in water at 110 to 112° F. for one hour at fortnightly intervals between July 26 and October 19, in 1937. After they had been removed

TABLE I

THE EFFECT OF A ONE-HOUR IMMERSION OF IRIS BULBS IN WATER AT 110 TO 112° F. ON BULB AND NEMATODE SURVIVAL AS INFLUENCED BY THE DATE OF TREATMENT

Variety	Date of treatment	Planted weight, gm.	Harvested weight, gm.	Percentage infested with bulb nematode
Supreme	Check	1000	837	94
	Check	1000	602	96.2
	26 July	1000	1340	0
	9 August	1000	1436	0
	23	1000	1133	0
	9 September	1000	626	0
	20	1000	336	0
	19 October	1000	184	0
Imperator	Check	1000	1625	0*
	Check	1000	1454	0
	Check	1000	1410	0
	26 July	1000	1733	0
	9 August	1000	1684	0
	23	1000	1463	0
	9 September	1000	1335	0
	20	1000	1320	0
	19 October	1000	640	0
Wedgewood	Check	1000	1625	0
	Check	1000	1485	0
	Check	1000	1442	0
	26 July	1000	1757	0
	9 August	1000	1671	0
	23	1000	1465	0
	9 September	1000	1367	0
	20	1000	1350	0
	19 October	1000	670	0

* *The Imperator and Wedgewood bulbs were included to ascertain their physiological response to hot water. The planting stock was slightly infested but no nematodes could be found in the harvested crop, irrespective of treatment.*

from the hot water bath, they were immersed in a 0.0015% suspension of mercury as methyl mercury nitrate* for 30 min., then placed in storage. All parcels were planted in the field on November 4. The immersion in an organic mercury suspension was deemed essential as a protection against *Penicillium* rots, which normally cause considerable injury; and susceptibility to *Penicillium* injury is increased by hot water treatments.

The results are given in Table I. It is readily seen from the weight of the bulbs harvested that injury occurs unless the bulbs are lifted early and immersed shortly after. In all three varieties, injury occurred after August 23 and became progressively greater as the immersion periods were delayed. It is apparent that a one-hour treatment destroys the majority of the nematodes. No living nematodes were recovered from any lot immersed for 60 min. at 110 to 112° F.

* *Sold under the trade name of "Leytosan", with mercury equivalent to 1.5%.*

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MICROBIOLOGICAL STUDIES OF APPALACHIAN PODSOL SOILS

IV. THE DECOMPOSITION OF GLUCOSE IN SOILS PREVIOUSLY TREATED WITH AMENDMENTS[1]

By P. H. H. Gray[2] and C. B. Taylor[3]

Abstract

A study has been made of the decomposition of glucose in two cultivated podsol soils that had previously been treated with alkaline amendments. Limestone increased, and sodium carbonate decreased, the amount of carbon dioxide produced from the control samples; limestone increased the rate of evolution both in control samples and in samples receiving glucose. The total amount of carbon dioxide produced was increased by limestone in combination with sodium carbonate but not by limestone alone. The numbers of heterotrophic bacteria developing with glucose were stimulated by limestone.

Introduction

It has recently been shown (3) that the addition of alkaline amendments to podsol soils increased total microbial activity, nitrification, and the numbers of bacteria. The significant increases in the three biological factors studied were, by the average of treatments, 62, 181, and 195% respectively. These values are derived from the average of the percentage increases found, less the percentage required for a significant difference, in 10 of the 11 treated plots. The increases suggested that the treatment of field soil with calcium oxide, sodium carbonate, and sodium hydroxide released a considerable amount of readily available food material for the soil micro-organisms. The material released must have contained carbonaceous as well as nitrogenous substances, since the carbon dioxide output as well as the activity of the heterotrophic and the nitrifying bacteria was increased. Some of the carbonaceous material may have been derived from the incompletely decomposed plant residues, and some from that part of the soil organic matter that includes the cells of micro-organisms. Shaw and McKibbin (5) have determined the amount of soluble organic matter that such treatments will liberate from podsol soils under laboratory conditions; it is not known, however, what proportions of the freed material will serve as sources of energy and food for soil organisms.

The ratio of organic carbon to total nitrogen in these soils is about 15 : 1. As the relative increase in activity was least in terms of the soil's total microbial complex, it would seem reasonable to assume that there was relatively less carbonaceous than nitrogenous material liberated by those treatments. It would appear, therefore, that the carbonaceous residues of the soil are in a

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Contribution from the Faculty of Agriculture, McGill University, Macdonald College, Que., Canada. Macdonald College Journal Series No. 115. This paper is the fourth in a series dealing with part of the work of the Soil Fertility Committee of Macdonald College.
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form not readily made available, and only partially so after treatment with organic matter solvents such as sodium carbonate. It has been shown that the addition of nitrate of soda alone does not increase microbial activity or bacterial numbers in these soils (3).

It seemed, therefore, that an understanding of the nature of the processes arising from such treatments could be obtained by determining the intensity of microbial activity after adding an easily available carbon compound, such as glucose, to soils previously treated with amendments.

Experimental

Numbers of Micro-organisms

The soils selected for study were taken in May 1937 from plots under experiment in the vicinity of East Clifton and Sawyerville, Compton County, Que. The plots were situated on two farms, and had been treated with limestone, sodium carbonate, and a combination of these two amendments, as reported in a previous paper (2). The treatments were applied in October 1935. Their effects on the numbers of bacteria (with actinomyces) and fungi were studied in five samplings during the summer of 1937, when the plots were under clover and timothy grass. The average numbers of micro-organisms in the samplings, determined by plating with Thornton's medium, with soil extract glucose agar for bacteria and actinomyces, and with the latter medium made acid with tartaric acid for the fungi, were as shown in Table I.

TABLE I

Numbers of micro-organisms in soils treated with amendments

Soil treatment	Soil *R*		Soil *T*	
	Thornton's medium	Soil extract agar	Thornton's medium	Soil extract agar
	Bacteria and actinomyces, millions per gm.			
Control	6.93	13.96	12.47	24.18
Limestone	12.04	26.86	35.43	35.74
Sodium carbonate	13.43	27.20	28.28	31.96
Both amendments	14.63	31.80	32.39	35.04
	Fungi, thousands per gm. (av. of 3 samplings)			
Control	—	125	—	147
Limestone	—	88	—	133
Sodium carbonate	—	105	—	115
Both amendments	—	103	—	132

The results given in Table I show that with both mediums used the bacteria were greatly increased by the treatments, though these had been applied 18 to 23 months previously. The sodium carbonate was as effective as the

limestone; both amendments together appear not to have caused any increase over the numbers given by each one separately. The effects of sodium carbonate had thus lasted through two seasons, as shown in the earlier work (3). Soil *T* supported the denser microflora of the two, as shown by comparing the numbers in the control plots. This difference, as previously pointed out (2), was probably due to the fact that this soil had been in the virgin state only two years before. The addition of the amendments to this soil caused a greater increase in the numbers of bacteria growing in the more selective medium, than in the soil extract agar.

The fungi were depressed by the treatments.

Nitrifying Capacity

Samples from these plots were also used to inoculate a mineral salt medium, in order to determine if the treatments had stimulated the nitrifying bacteria to develop a greater capacity to oxidize ammonium sulphate under controlled conditions in the laboratory. Since the nitrifying bacteria prefer a neutral or alkaline environment to enable them to oxidize ammonium compounds most rapidly, it was expected that these treatments might be found to stimulate the rate of nitrification.

The pH values of fresh samples from these plots were determined by the electrometric method (by Mr. H. L. Fletcher) and were found to be as follows:

Treatment	Soil *R*	Soil *T*
Nil	4.98	4.70
Limestone	6.18	6.67
Sodium carbonate	5.63	5.42
Both amendments	6.96	5.71

The medium used for cultivating the nitrifying bacteria was composed of the following salts (1): K_2HPO_4, 1.0 gm.; $MgSO_4 . 7H_2O$, 0.5 gm.; NaCl, 2.0 gm.; $FeSO_4$, 0.4 gm.; $(NH_4)_2SO_4$, 1.0 gm.; water, 1000.0 gm. The medium was dispensed in 25-ml. quantities in Erlenmeyer flasks of 125 ml. capacity, and sterilized. Magnesium carbonate was sterilized in 0.2-gm. quantities in tubes and added to the sterile salts. Three flasks of medium were inoculated with 1 gm. of each soil, three with a garden soil over limestone for comparison, and three were kept as sterile controls. The cultures were incubated at 28° C. and tested at intervals for nitrites and nitrates. Nitrites were tested for by means of Trommsdorf's reagent. After the nitrites had disappeared, the cultures were tested for nitrates with diphenylamine in sulphuric acid. The results are shown in Table II. The oxidation of ammonium sulphate proceeded more quickly, in both soils, with samples from the plots that had received sodium carbonate than with samples from the control plots. The soil with the higher bacterial numbers, Soil *T*, was the more active, though slightly more acid, and treatment with limestone brought it up

to the level of a rich garden soil. The combination of sodium carbonate with limestone stimulated the bacteria in Soil *R* more than did limestone alone. This test would seem to confirm the view that the treatments with sodium carbonate had released nitrogenous material for the use of the nitrifying

TABLE II

NITRIFICATION OF AMMONIUM SULPHATE IN SOLUTION CULTURES

Soil	Treatment	Duration of experiment, days												
		5	8	11	14	18	22	24	27	31	35	38	41	51
R	Nil	0	0	0	0	0	0	±	±	±	+	+	∓	∓
	$CaCO_3$	0	0	0	0	±	+	+	+	+	+	+	∓	X
	Na_2CO_3	0	0	0	0	±	±	±	+	+	+	+	+	+
	$CaCO_3$ + Na_2CO_3	0	0	+	+	+	+	+	+	∓	X			
T	Nil	0	0	0	0	+	+	+	+	+	+	+	X	
	$CaCO_3$	0	+	+	+	+	+	+	X					
	Na_2CO_3	0	0	0	±	+	+	+	+	+	X			
	$CaCO_3$ + Na_2CO_3	0	+	+	+	+	+	∓	X					
Garden	—	0	+	+	+	+	∓	X						

0 = *nitrite not yet formed;* ± = *nitrite reaction slight;* + = *nitrite reaction strong;* ∓ = *nitrites decreasing;* X = *nitrites absent, nitrates present.*

bacteria in the soil, enabling them to develop higher numbers and to oxidize fresh material more effectively. The effect of the limestone was probably limited to reducing the soil acidity, which could also enable these bacteria to become more active. It was expected, therefore, that fresh carbonaceous material would be more rapidly decomposed in the presence of some of the released nitrogenous material. The reduction of acidity which gave rise to higher numbers of heterotrophic bacteria might also be assumed to stimulate the utilization of glucose.

DECOMPOSITION OF GLUCOSE

Samples from the plots at the two places had been allowed to dry rapidly in the air of the laboratory. The experiments began on July 13 (Soil *T*) and July 27 (Soil *R*), 1937. The treatment of the samples and the methods used have been described in a preceding paper (4).

The amounts of glucose recovered from the incubated samples are shown in Table III; the amount added was 32.1 mg. per 10 gm. of moist soil. After the glucose had disappeared, the pH value of the "nil" samples, determined colorimetrically, was 7.0 in both soils; that of the "$CaCO_3$" samples was from 7.5–8.0.

The carbon dioxide was collected at intervals for 10 days. The total amounts at the end of that time are given in Table III. Previous treatment with the limestone appears to have developed a more active microflora

in Soil *R*, for the increased evolution of carbon dioxide is seen in the control cultures as well as in those with glucose. The treatments do not appear to have been effective in the soil *(T)* more recently brought into cultivation, though activity in this soil was somewhat greater in the control cultures than in those of Soil *R*.

TABLE III

Soil		Treatments			
		Nil	$CaCO_3$	Na_2CO_3	Na_2CO_3 + $CaCO_3$
		Glucose recovered, mg.			
R	At start	32.3			
	Day 1	24.9	26.8	24.6	22.9
	Day 2	8.0	Nil	5.5	1.8
	Day 3	Nil	Nil	Nil	Nil
T	At start	28.5			
	Day 1	23.2	17.0	19.8	18.6
	Day 2	Nil	Nil	Nil	Nil
	Day 3	–	–	–	–
		Total CO_2, mg. per 100 gm. soil			
R	With glucose	418	435	410	498
	Control	91	114	89	147
	Minus control	327	321	321	351
T	With glucose	483	492	456	483
	Control	161	188	139	156
	Minus control	322	304	317	327

The amounts of gas evolved from the glucose were about equal for all treatments, and were similar in both soils, except that that from "limestone" in Soil *T* was somewhat less, and that from "both amendments" in Soil *R* was increased; the differences may, however, be significant. The utilization of fresh energy material by a restricted microflora appears to have masked the differences brought about by the amendments.

The results suggest that the normal soil microflora had been stimulated by limestone, which effected a change in acidity only, and that any earlier benefits of the sodium carbonate, such as were reported previously (3) in studies of similar soils, were no longer in evidence.

The rates of carbon dioxide production were determined in the manner described in the previous paper (4); the results are given in Table IV, in which the values represent milligrams of carbon dioxide per 100 gm. of moist soil per hour.

The limestone appears to have remained more effective than the sodium carbonate; the latter stimulated activity only in Soil *T*, which contained

more of the residues remaining in a state of partial decomposition, and had developed higher numbers of bacteria. The earlier high rates in Soil *T* correspond with the earlier disappearance of the glucose. The numbers of

TABLE IV

CARBON DIOXIDE PRODUCTION, MG. PER 100 GM. SOIL PER HOUR

Soil	Time, hr.	Treatment			
		Nil	$CaCO_3$	Na_2CO_3	Na_2CO_3 + $CaCO_3$
R					
Cultures with glucose	23.25	0.86	2.46	2.20	3.40
	36.75	5.39	6.58	5.22	7.19
	45.25	6.72	8.47	6.66	6.03
	69.25	4.04	2.46	3.48	3.56
	143.75	1.28	1.12	0.95	1.29
	239.75	0.79	0.78	0.81	0.93
Control cultures	23.25	0.19	0.65	0.48	1.00
	36.75	1.18	1.73	1.13	2.13
	45.25	1.47	2.27	1.44	1.78
	69.75	0.88	0.64	0.75	1.05
	143.75	0.28	0.30	0.21	0.38
	239.75	0.17	0.20	0.17	0.27
T					
Cultures with glucose	23	—	4.54	3.48	4.53
	35	5.16	8.28	7.86	8.11
	44.5	—	6.35	6.75	7.40
	68.5	3.68	2.42	1.94	1.81
	140.5	1.52	1.34	1.31	1.31
	238.5	0.70	0.75	0.78	0.74
Control cultures	68.5	—	1.41	0.93	1.15
	238.5	0.68	0.54	0.44	0.46

TABLE V

BACTERIA AND ACTINOMYCES, MILLIONS PER GRAM

Time, days	Soil *R*				Soil *T*			
	Nil	$CaCO_3$	Na_2CO_3	$CaCO_3$ + Na_2CO_3	Nil	$CaCO_3$	Na_2CO_3	$CaCO_3$ + Na_2CO_3
	Cultures with glucose				Cultures with glucose			
At start	1.51	2.17	1.54	3.55	1.40	2.47	1.47	2.08
1	6.35	146.80	60.80	207.10	16.43	104.30	15.31	34.40
2	112.60	157.40	102.00	176.10	25.07	88.31	63.06	90.93
3	107.40	168.60	131.50	174.50	108.30	141.10	83.37	196.30
4	121.80	194.30	107.00	162.25	22.82	151.00	52.65	101.60
6	96.23	172.60	39.68	82.24	39.08	125.70	45.04	82.94
	Control cultures				Control cultures			
2	17.39	50.59	59.91	53.46	3.62	12.54	16.55	13.24
4	25.28	50.01	26.45	51.94	16.01	29.31	33.66	36.97

bacteria and actinomyces, in soil extract glucose agar, were as shown in Table V. The colonies in the samples treated with glucose were, after the start, bacterial only. The high numbers in the control cultures were caused by the long period of drying that preceded the remoistening of the samples.

The bacteria in the *R* cultures reached their highest numbers 24 hr. earlier than those in the *T* cultures, although the glucose was decomposed more rapidly in the latter. The limestone, both alone and with sodium carbonate, caused the greatest increases. In both soils the higher numbers with the limestone were associated with increased rates of carbon dioxide production from the samples receiving glucose.

The beneficial effects that may have arisen from the organic matter released by the sodium carbonate shortly after the soils had been treated were not shown as clearly as those derived from the treatment with limestone.

Acknowledgments

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VIBRIO AMYLOCELLA, N. SP., A SOIL ORGANISM THAT DECOMPOSES CELLULOSE AND PRODUCES GLUCOSE FROM STARCH[1]

By P. H. H. Gray[2]

Abstract

Vibrio amylocella, n. sp., isolated from soil, attacks cellulose, starch, dextrin, and sugars. It does not grow in mediums containing beef extract. It can obtain its nitrogen from peptone, amino-acids, and mineral salt compounds, when supplied with a suitable source of carbon. Glucose is formed from starch and dextrin in liquid cultures, and accumulates in a starch medium containing ammonium chloride, but is utilized completely when potassium nitrate is supplied. The accumulation of glucose is related to the development of acidity, which renders the organism non-viable. Calcium carbonate depresses diastatic action and enables the organism to utilize the glucose formed.

The diastatic enzyme of *V. amylocella* is more active apart from the organism than in its presence. The organism is more active in starch hydrolysis than some common diastatic organisms.

Introduction

The decomposition of plant residues by soil bacteria and other fungi depends for completeness upon a great variety of micro-organisms, among which different groups play essential roles. Structural material such as cellulose, or storage products such as starch, are decomposed to yield ultimately carbon dioxide and water; the intermediate stages of the dissimilatory process in soil are not known but are assumed to be those found when such materials are acted on by micro-organisms under laboratory conditions. In such studies, cellulose and starch have given rise to sugars, fatty and other acids, acetone and alcohols. The bacteria described as the agents in that type of decomposition are mainly anaerobes, producing acids and alcohols from the sugars formed. Reference to such organisms and their activities are given by Thaysen and Galloway (16).

The production of sugars in soil has to be assumed, for they are so readily decomposed by aerobic as well as by anaerobic bacteria that they are not found as intermediate products in agricultural soil. Since, however, the sugars provide an easily utilizable source of carbon and energy, it would appear worth while to isolate and study specific micro-organisms that produce them from cellulose or starch. The present study concerns a bacterial organism that attacks cellulose and produces glucose from starch.

Among the bacteria that have been reported to produce glucose from starch are *B. suaveolens* (13), *B. anthracis* (10, 11), *B. maydis* (3), species of *Glycobacter* (18), *B. citreus cadaveris* and *B. cloacae* (8), *Granulobacter pectinovorum* (15), a coccus, not named (14), a cellulose-decomposing organism, not named (12), and *Streptobacterium plantarum* (4).

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Glucose was shown to be present, by the osazone test, in the cultures of *Granulobacter*, of the cellulose-decomposing organism, and of the *Streptobacterium*. Napias (11) states that *B. anthracis* produces sugar whose nature she did not determine, but accepted as being partly glucose on the basis of work by Fernbach, who later reported maltose was produced by an extract of cells of *Tyrothrix (Bacillus) tenuis* (5); the maltose was changed to dextrose and then to dihydroxyacetone. In Napias' work the sugar was reported to have been destroyed before all of the starch had been hydrolyzed. Cavazzani (3) and Wollman (18) give figures for the yield of sugar (calculated as glucose); the former quotes a yield of 23 mg. of glucose from 100 mg. of starch in 48 hr. In Wollman's work, *Glycobacter proteolyticus* produced 2.5 gm. of sugar (calculated as glucose, but stated to be a mixture of glucose and maltose) from 6 gm. of starch. *Glycobacter peptolyticus* appears to have been less active.

The organism that forms the subject of the present study differs from any previously described species of the Schizomycetes in its cultural characters and biochemical activities. On the basis of its morphology, and from its ability to attack cellulose and starch in synthetic mediums, the binomial *Vibrio amylocella* is proposed for it.

Isolation and Cultural Characters

Vibrio amylocella, n. sp., was isolated in February 1938, from manured garden soil, of the brown-earth type over limestone. The sample contained a large amount of fibrous débris of grass roots. A suspension of the soil was inoculated into cellulose medium composed of a strip of filter paper partly immersed in 10 ml. of a mineral salt solution, with potassium nitrate 0.2%. The paper became disintegrated chiefly at the air-liquid interface. Actively motile bacteria of the curved rod form were isolated by means of an agar medium containing soluble starch. Colonies were light greenish yellow. Pure cultures decomposed filter paper cellulose and were strongly diastatic.

The organism was unable to grow in ordinary mediums containing beef-extract. Peptone could support growth when supplied as a source of nitrogen in starch hydrolysis, in the decomposition of cellulose, or with certain other carbonaceous compounds as sources of energy. Gelatin is slowly liquefied if a suitable source of carbon, such as starch, dextrin, or glucose, is present.

The organism, of which only one strain has been isolated, is aerobic; it does not utilize cellulose or starch in synthetic mediums from which atmospheric oxygen has been excluded. The young cells on starch agar are short, thin, curved rods, measuring about 2 to 3 μ long, or longer preceding division in the adolescent phase, by 0.6 μ broad. The motile cells from a culture on starch agar, after 24 hr. at 30 to 35° C., have from one to four polar flagella (Fig. 1). Capsules can be demonstrated by Muir's method, but are too tenuous to be seen in films prepared with nigrosin. In its morphology and manner of attacking cellulose, it thus resembles *Vibrio agarliquefaciens* (7), and some of the species of cellulose-decomposing vibrios studied by Kalnins (9). It differs from those organisms in that it does not grow in ordinary

mediums, nor liquefy agar. Among other mediums which did not support any growth were peptone broth, tryptophane broth, litmus milk, dextrose and lactose agars (both containing beef-extract), potato slant and block, potato dextrose agar, peptone glycerol broth, and glycerol, mannitol, inulin, and glycogen in synthetic mediums.

FIG. 1. *Vibrio amylocella, showing flagella, from a 24-hr. culture at 33° C. on starch agar. Stained by Gray's method (6). ×750. (Contact print from camera lucida drawing.)*

The action on cellulose is rapid; motile cells are visible in a wet mount from a culture after less than 24 hr. at 30° C., though disintegration of the paper may not then be visible. In some cultures, with potassium nitrate as the source of nitrogen, the paper begins to disintegrate within 24 hr. The organism decomposes the paper at temperatures from 15° C. to 37° C.; at the latter temperature the attack on the paper is more rapid than at 30° C. It does not grow at 41 to 42° C. Reducing substances have not been found in cellulose cultures. The amount of cellulose decomposed was ascertained by growing the organism in a mineral salts medium with potassium nitrate, with a round of filter paper weighing 0.55 gm. supported at the air-liquid level on glass beads in a 500-ml. Erlenmeyer flask; after one month the culture was filtered through a weighed paper; the loss in dry weight was 39%.

The organism develops catalase in a medium in which cellulose or dextrose has been attacked.

Methods

In the experiments, the results of which are reported below, the starch used, except where otherwise stated, was the Analar quality soluble starch from British Drug Houses, Limited (1). It was prepared for incorporation into

mineral salt or peptone mediums by dissolving cold water suspensions in boiling water. The suspension, in a small beaker, was poured a little at a time into the water, and the small beaker washed with more cold water. After boiling for 2 or 3 min., the opalescent liquid was mixed with the mineral salts or peptone solution, and the beaker washed with water into the mixture. With the higher percentages of starch, a small proportion of the starch adhered to the beaker, but the loss was probably negligible. Examination under the microscope showed that there were no whole grains of starch in the opalescent fluid.

The figures given below as "starch, per cent" added to a medium represent the amount of starch as weighed with the original moisture content, which was found to be 8% in the sample used. The values quoted for "glucose, mg. per 100 mg. of starch" do not allow for the small fraction of starch that did not enter the medium. The values include the allowance for moisture content. The results in terms of glucose can be accepted as approximate only, and are based on the ratio of dry weight of starch 91: glucose 100. Since loss of water by evaporation is a factor that has to be considered, at least in cultures that were incubated for the longer periods of time, some of the values reported may be too high. The results are presented, however, not as absolute values, but as comparative in any one set of cultures.

Reducing sugars were determined, in cultures filtered through a No. 3 Whatman paper, by Bertrand's method (2). By this method it was found possible to recover as much as 99.9% of a weighed amount of pure anhydrous glucose (dextrose) in solution, in repeated tests. The only modifications adopted in the method were to make the asbestos filter in a Gooch crucible, and to employ a Schuster bottle for small amounts of culture in the copper reduction process. The washings from the beaker, or bottle, as well as those from the filter, were tested with litmus paper until no trace of acid was present. Duplicate determinations with filtrates from cultures were in close agreement. In the results quoted in this paper, no allowance has been made for sterile control mediums, since it was found that the filtrates from these, after treatment with the reagents, changed colour with one drop of the permanganate solution.

Sources of Nitrogen

Suitable sources of inorganic nitrogen were found by inoculating mineral salt mediums, in 6 in. by $\frac{3}{4}$ in. tubes, containing nitrogen compounds as shown below, with carbon supplied as cellulose in the form of filter paper strips, or as 0.2% starch. The medium contained the mineral salts recommended by Thornton (17) for a standardized agar medium used in counting soil bacteria (Table I). The amount of nitrogen salt added was calculated to give 28 mg. of N per 100 ml. of medium.

In the starch cultures, after 48 hr., iodine gave a blue colour in Mediums 3 and 7; the brown-red colour of erythrodextrin was developed in the others.

After 72 hr. all of the starch and erythrodextrin had been destroyed in the mediums marked ++; erythrodextrin was still present in Medium 7, but later disappeared. Calcium nitrate did not support any growth with either source of carbon; the ammonium phosphate did not support growth in the cellulose medium. Nitrite was found in the cultures of both series supplied with potassium and sodium nitrate.

TABLE I

Medium	Source of nitrogen	Per cent	Growth with	
			cellulose	starch
1	KNO_3	0.200	++	++
2	$NaNO_3$	0.170	++	++
3	$Ca(NO_3)_2$	0.164	0	0
4	$(NH_4)_2SO_4$	0.132	+	++
5	NH_4NO_3	0.080	+	++
6	NH_4Cl	0.107	+	++
7	$NH_4H_2PO_4$	0.115*	0	+

** Amount added in error for 0.230.*

Reducing sugar was found in the starch cultures having ammonium chloride; the phenylhydrazine test yielded glucosazone only.

The comparative values of different sources of nitrogen on diastatic action were determined by the plate method. A comparison was made between two different sources of mineral nitrogen. Plates of starch agar containing 0.1% potassium nitrate, and 0.2% ammonium chloride, were inoculated on the surface and incubated at 30° C.; the diameters of the colonies and of the diastatic zones were as shown in Table II.

Ammonium chloride was evidently preferred to potassium nitrate, though to a small extent and only after six days. This was confirmed in an experiment, reported below, in which the activity of this organism was compared with

TABLE II

Plate	KNO_3		NH_4Cl	
	Colony diam., mm.	Zone diam., mm.	Colony diam., mm.	Zone diam., mm.
	3 days			
a	1.5	20	3	23
b	3	27	5	23
c	4	27	5	28
	6 days			
d	3	30	3	41
e	3	34	3.5	39
f	3	35	4	40

that of others (Table X). The effect of different amounts of mineral-salt nitrogen was also tested. Plates were prepared with 10 ml. of agar with 0.2% starch, found to be the concentration giving the greatest activity in solution cultures, and ammonium chloride in the amounts shown in Table III. After three and six days duplicate plates were treated with iodine and the diameters of the clear zones measured.

TABLE III

EFFECT OF DIFFERENT AMOUNTS OF AMMONIUM CHLORIDE ON DIASTATIC ACTIVITY

NH_4Cl, %	Diameter of clear zone, mm.			
	3 days		6 days	
	Plate a	Plate b	Plate a	Plate b
0.025	22	23	36	37
0.05	18	20	35	32
0.10	19	18	38	34
0.20	18	18	37	36
0.40	22	22	38	40

There was no close agreement between the diameter of the colonies and the diastatic activity; thus, on the sixth day, with 0.2% ammonium chloride the colonies measured 5 mm. and 3 mm., while with one-fourth of that concentration the diameter of the colonies was 7 mm.; also, the colonies with 0.2% had not increased in diameter after the third day, though the diastatic zone had doubled in diameter. The concentration of ammonium chloride, within the range tested, had no clear-cut effect on size of colony or diastatic activity. It would appear that enzymic activity was not directly related to size of colony at the times the tests were made.

The effect of peptone, in comparison with ammonium chloride and potassium nitrate, is shown later, in Table X. Another test was made with glycine, *d*-alanine, and asparagine as sources of nitrogen, in comparison with ammonium chloride and with no added nitrogen, in starch agar plates and slant cultures. The amount of nitrogen supplied by each amino-acid was equivalent to that given by the salt, namely 52.8 mg. The diameters of the colonies and of the clear zones in the plates, after 5 days, were as shown in Table IV.

TABLE IV

Nitrogen source	Amount, %	Colony diameter, mm.		Zone diameter, mm.	
		a	b	a	b
Nil	—	1.0	1.0	16	13
NH_4Cl	0.200	2.5	3.0	29	27
Glycine	0.284	No growth		Trace only	
Alanine	0.300	1.5	2.3	17	21
Asparagine	0.250	3.0	—	27	—

The glycine apparently prevented growth, which was appreciable without added nitrogen.

The growth in the slanted agar containing starch was about equal in amount with asparagine and ammonium chloride, less with alanine, and only slight with no added nitrogen*. At the same time, plates and slant cultures were prepared with these materials but without starch; there was some growth with the alanine and asparagine, but none with the glycine.

Viability; Effect of Calcium Carbonate

Early in this study it was noted that some cultures were no longer viable after about 10 days at 30° C. This was found to be true of cultures containing ammonium chloride, but not of those containing potassium nitrate. The former were found to develop acidity, while the latter remained neutral or became slightly alkaline. A series of cultures was therefore prepared to determine how long the organism remained viable in a starch medium with ammonium chloride. Cultures of 10 ml. in 6 in. by $\frac{3}{4}$ in. tubes were incubated at 20, 25, and 30° C.; the medium was composed of 0.2% starch, 0.1% ammonium chloride, inoculated in quadruplicate, one pair at each temperature having 0.1% calcium carbonate. Transfers were made after 7, 9, 11, 14, 19, and 26 days into starch or cellulose mediums, by means of a triple-loop. The number of days after which viable cells were still present was as follows:

At	20°	25°	30° C.
Without calcium carbonate	26+	14	11
With calcium carbonate	26+	26+	26+

The test was not continued after the 26th day.

The effect of calcium carbonate was also tested in plate cultures; the medium was the same as that used in the test for viability, with the addition of 1.0% agar. The plates were treated with iodine and the clear zones found to measure as shown below; each value represents the mean diameter of the clear zone of two cultures, in millimeters:

	4 days	7 days
Without calcium carbonate	34	44
With calcium carbonate	29	34

Diastatic activity was thus depressed in the presence of calcium carbonate. It was also found that, in addition to prolonging viability, calcium carbonate enabled the organism to utilize the glucose formed. The cultures grown at 20° C., in the test for viability, were later used for a determination of reducing power. In the culture without calcium carbonate, a large amount of cuprous oxide was obtained in the reduction test on the 35th day; none was obtained from the culture with calcium carbonate.

* *The organism did not grow in solution cultures containing starch without added nitrogen.*

Effect of Potassium Nitrate

As alkaline conditions enabled the organism to utilize the glucose that accumulated in mediums with ammonium chloride but without calcium carbonate, a test was made to determine the fate of the glucose in cultures containing 0.2% starch and 0.1% potassium nitrate, because the utilization of this salt rendered the medium alkaline. The cultures were grown in 50-ml. quantities in Erlenmeyer flasks of 125 ml. capacity. Tests were made, at the times shown in Table V, for disappearance of starch and for the amount of sugar formed, calculated as glucose; the total numbers of bacteria were counted by means of a haemocytometer.

TABLE V

DECOMPOSITION OF STARCH, WITH NITROGEN AS POTASSIUM NITRATE

Hours or days	Iodine test	Glucose, mg. per 100 mg. starch	Bacteria, millions per ml.
12 hr.	Starch	4.2	134
47 hr.	Starch	6.0	200
63 hr.	Erythrodextrin	3.6	420
70 hr.	Nil	12.9	800
86 hr.	—	27.8	880
7 days	—	5.4	2400
12 days	—	Nil	—

It is evident that with potassium nitrate as the source of nitrogen the glucose formed was utilized by the increased numbers of bacteria after the 86th hr. It would also appear probable, from a comparison with results obtained in tests with glucose, reported below, that glucose was being used to some extent during the earlier stages.

Although the growth of the organism proceeded while glucose was beginning to accumulate, it is not reasonable to conclude that energy was being derived from the hydrolysis. From the test with potassium nitrate as the source of nitrogen, it seems conclusive that the sugar was the source of energy after the 86th hr. With ammonium chloride supplying nitrogen, the accumulation of the sugar may be due to the progressive dissolution of the cells in an acid medium.

Evolution of Carbon Dioxide

An experiment was made to ascertain when the metabolic activity of the organism was at its highest in cultures containing ammonium chloride. Flasks of 500 ml. capacity, containing 200 ml. of 0.2% starch and 0.2% ammonium chloride, were inoculated and connected to tubes of baryta for the absorption of carbon dioxide by aspiration of air previously freed of that gas. Two tubes, each with 20 ml. of 0.105 *N* barium hydroxide, were used in series to collect the carbon dioxide from each culture. The whole apparatus was placed in an incubator at 28 to 30° C. The carbon dioxide was determined at intervals as shown in Table VI.

TABLE VI

Hours or days	Carbon dioxide, mg.		Glucose, mg. per 200 ml.
	Amount	Per hour	
25 hr.	69.6	2.78	
30 hr.	20.2	4.05	
35.25 hr.	11.9	2.27	
48.25 hr.	14.3	1.12	24
108 hr.	12.8	0.21	—
5 days	—	—	48
13 days	—	—	89
Total in 4.5 days	128.8		

The amount passing over into the second tube is included in the value quoted for the 25th hr.; the amount collected at the 108th hr. in the second tube was only 5.7 mg., which has been omitted from the total.

The organism evidently developed its maximum metabolic activity before the 48th hr., when there was no trace of starch or erythrodextrin. The amount of carbon dioxide collected in 48 hr. was equivalent to 79 mg. of glucose oxidized. The pH of the medium was 5.2 at the 108th hr.

The fact that sugar was not found until metabolic activity had slowed down would suggest that, under these conditions, either (i) the organism obtained its energy by the hydrolysis, and the production of glucose was due to the release of the enzyme dextrinase from dying or dead cells, or (ii) some glucose was produced during the period of greater activity in an amount sufficient to supply energy (by oxidation) to the extent shown, the hydrolytic enzyme being restricted by the aeration, so that the glucose accumulated to an extent relatively less than it did in non-aerated cultures incubated for the same length of time. The former alternative would seem unlikely, since the possession of an enzyme that leads to the production of energy material from a substrate pre-supposes that the organism will use all or part of the energy so produced; the accumulation (or non-utilization) of part of the energy source set free by the enzyme may, nevertheless, be due to the cause stated. In regard to the latter alternative, this would appear to receive support from the fact that the organism utilizes glucose in the presence of ammonium chloride, as shown below in the section headed "Other sources of carbon".

Concentration of Starch

The action of the organism on different concentrations of starch was tested with plate cultures and with solution cultures. Plate cultures were prepared with starch in the concentrations shown in Table VII and with 0.2% ammonium chloride added to mineral salts agar. The relative concentrations of starch and nitrogen were the same as those used in the test in which different concentrations of ammonium chloride were used. The plates were incubated at 28° C., and tested with iodine on the third and sixth days.

TABLE VII

DIASTATIC ACTION OF *V. amylocella* IN AGAR WITH DIFFERENT CONCENTRATIONS OF STARCH

Starch, %	Diameter of clear zone, mm.			
	3 days		6 days	
	a	b	a	b
0.1	20	—	36	42
0.2	21	—	40	43
0.4	18	17	33	38
0.8	15	14	28	27
1.6	8	8	25	—

At the same time, cultures on peptone agar with 1.0% starch were prepared; the clear zones measured 7 to 8 mm. on the third day and 20 to 21 mm. on the sixth.

It would appear that, in the earlier stages, a concentration of starch over 0.2% depressed diastatic action; on the sixth day, however, although the lower concentrations developed the greater areas of diastatic activity, the relative increase over that shown at the third day was greatest in the culture having the highest concentration. The mineral nitrogen was preferred to the peptone, the concentration of which was 0.5%.

Two series of cultures were made for testing the activity in solution cultures. In both series, different concentrations of starch were used in Erlenmeyer flasks of 125 ml. capacity, containing 50 ml. of medium; nitrogen was supplied as ammonium chloride. The cultures were incubated at 28 to 30° C. In the first series, portions were removed aseptically at intervals for the determination of reducing sugar (glucose). The results shown in Table VIII include three only of the concentrations used.

TABLE VIII

AMOUNTS OF GLUCOSE FORMED FROM DIFFERENT CONCENTRATIONS OF STARCH

Hours or days	Glucose, mg. per 100 mg. starch		
	0.4% starch	0.8% starch	1.2% starch
60 hr.	25	23	14
90 hr.	78	51	54
110 hr.	73	48	43
6 days	—	—	56
8 days	82	69	70
12 days	—	73	—

The experiment showed that the higher concentrations of starch reduced the diastatic activity in the early stages. This was further confirmed in the second series of cultures, which were supplied with starch, 0.2% and 1.0%.

Eight flasks of each medium, having a pH value of 7.2, were inoculated with one drop each of a suspension of the organism. At intervals of five days, duplicate cultures were taken for the determination of the pH value by the colorimetric method, and for glucose; the results are shown in Table IX.

TABLE IX

Culture	0.2% starch				0.1% starch			
	pH		Glucose*		pH		Glucose*	
	a	b	a	b	a	b	a	b
Day 5	5.7	5.7	49	44	5.7	5.7	22†	25†
10	5.8	5.7	52	52	5.5	5.7	53	58
15	5.5	5.5	52	56	5.2	5.3	60	62
21	5.6	–	54	–	5.2	–	63	–

* *Mg. per 100 mg. of starch supplied.*
† *A trace of erythrodextrin was found.*

The cultures were not viable on the 10th day, after which there was little change in the pH values or the amounts of glucose. The phenylhydrazine test with these cultures yielded only glucosazone.

Action of the Enzyme

The action of the enzyme in the absence of proliferating or viable bacteria was tested in plate cultures and in solution cultures. Plate cultures of starch agar were incubated for two days, when the diastatic zone had developed sufficient area to permit blocks of agar to be cut and transferred to the surface of sterile agar, or into sterile liquid medium. Tests with iodine in parallel plate cultures, two days old, showed that the diastatic zone from which the blocks were cut contained no starch. The results of an experiment to compare the enzyme action with that of a colony are shown below. The plates with the blocks were incubated for three days.

Size of blocks cut at 2 days, mm.	Enzymic zone diameters, mm.	
	Plates with blocks, 3 days	Original plates, 5 days
3 by 4	37	36
8 by 10	42	44

It should be noted that the clear zones produced by the blocks had about the same diameters as those of the corresponding colonies, which were incubated for two days longer than the blocks. This may be interpreted as relatively greater activity of the enzyme both from its shorter period of contact with the starch, and from the fact that the blocks represented only a small percentage of the enzymic zone from which they were cut.

Blocks cut from enzymic zones in agar were also transferred aseptically to 10-ml. portions of liquid medium containing 1.0% starch and 0.2% ammonium chloride. Two large blocks (6 by 7 mm., and 7 by 8 mm.) and two small blocks (3 by 3 mm.) were each placed in a tube of medium. After 48 hr. at 30° C., there was no starch in the tubes having the larger blocks; after 72 hr., erythrodextrin was present in the medium containing the small blocks; this had disappeared on the 5th day. On the 13th day, the medium containing the largest block was found to contain about 40 mg. of glucose (allowance being made for evaporation of water). It would appear, therefore, that the enzyme developed by a portion of a colony may produce a considerable amount of glucose from soluble starch. Reducing sugar was not, however, found in plates of starch agar in which the organism, or its enzyme, had hydrolyzed the starch.

The action of the enzyme in the absence of proliferating bacteria was also tested in 10-ml. solution cultures having 1.0% starch and 0.5% ammonium chloride. After two days of growth at 30° C., starch was still present; some cultures were then treated with toluol, and others allowed to continue growth. After 24 hr. there were no viable cells in the tubes treated with toluol, while actively motile bacteria were seen in the control (untreated) cultures; erythrodextrin was present in both series. On the 5th day the erythrodextrin had disappeared, and on the 10th day the bacteria were not viable in the control cultures. The amount of glucose, in milligrams per 100 mg. of starch supplied, was determined at intervals; the results are shown below:

Days	Treated	Control
3	14	10
7	33	22
20	54	29

These results may be interpreted in two ways; the enzyme may be more efficient in the absence of living organisms, or the organism decomposed part of the glucose formed. As shown below, the organism is able to decompose a large amount of glucose in cultures having ammonium chloride as the source of nitrogen and glucose as the source of carbon.

Other Forms of Starch

Potato starch (Baker and Adamson quality, from the General Chemical Co., New York) serves as well as soluble starch as a source of carbon in a mineral salts medium. A 0.2-gm. portion of the starch was dissolved, as described previously, and mixed into 100 ml. of a medium with 0.1% ammonium chloride. Growth was visible, and motile cells were seen, within 24 hr. On the third day there was no trace of the colour of erythrodextrin by the iodine test. On the seventh day the cultures were acid, requiring 3.5 ml. of 0.1 *N* sodium hydroxide per 100 ml. to be neutralized. The amount of cuprous oxide obtained was equivalent to 57 to 58 mg. of glucose per 100 ml. of medium; (this value has not been adjusted to account for moisture or on

the basis of starch 91 : glucose 100). The phenylhydrazine test yielded crystals of glucosazone only. The yield of sugar from corn-starch (Kahlbaum) was 47 mg. in 10 days.

Comparison with Other Micro-organisms

Bacillus ruminatus, a common soil organism active in starch hydrolysis, and *Aspergillus oryzae* were compared with *V. amylocella* in plate cultures of starch agar. The organisms were each inoculated onto the surface of four plates of 0.2% starch agar, with nitrogen as 0.1% peptone, 0.1% potassium nitrate, and 0.2% ammonium chloride, and incubated at 30° C. After three and six days, duplicate cultures of each were treated with iodine, and the diameters of colonies and enzymic zones were measured.

The results are given in Table X, in which the figures represent the mean diameters of colonies and zones of complete hydrolysis (as shown by a clear white area) in two plates on each day, and also the ratios that the diameters of the colonies bore to the diameters of the clear zones. It should be noted

TABLE X

COMPARISON BETWEEN *Vibrio amylocella*, *Bacillus ruminatus*, AND *Aspergillus oryzae* IN PLATES OF STARCH AGAR

Diameters of colonies and enzymic zones in millimeters, and their ratios

Organism		Peptone		KNO_3		NH_4Cl	
		Colony	Zone	Colony	Zone	Colony	Zone
		Cultures 3 days old					
V. amylocella	Diam.	0.8	11.5	1.5	16.0	1.5	21.0
	Ratio	1	15.32	1	10.67	1	14.00
B. ruminatus	Diam.	4.0	7.0	3.0	5.0	2.5	4.0
	Ratio	1	1.75	1	1.67	1	1.60
A. oryzae	Diam.	23.0	24.0	9.0	10.0	20.0	21.0
	Ratio	1	1.00	1	1.01	1	1.00
		Cultures 6 days old					
V. amylocella	Diam.	2.0	16.0	2.25	15.0	2.25	31.0
	Ratio	1	8.0	1	6.8	1	13.8
B. ruminatus	Diam.	6.0	16.0	4.5	7.0	3.0	5.0
	Ratio	1	2.67	1	1.23	1	1.67
A. oryzae	Diam.	48.0	56.0	21.0	25.0	38.0	40.0
	Ratio	1	1.17	1	1.19	1	1.05

here that the actual diameters of zones of partial hydrolysis by *B. ruminatus* and *A. oryzae* were larger than those quoted, but the comparison was made with the complete hydrolysis effected by *V. amylocella*. *B. ruminatus* had three narrow zones: an inner white zone, a middle zone tinged with blue,

and an outer purple zone indicating the presence of erythrodextrin. *A. oryzae* developed also a blue-tinged wide border of incomplete hydrolysis. The ratio of colony to these outer enzymic zones was still smaller than that shown by *V. amylocella*, at the third day the ratios being, for *B. ruminatus* 1 : 4.33 (inner and outer zones together) and for *A. oryzae* 1 : 1.6.

The results from the plate cultures show that *V. amylocella* is considerably the most active organism, in proportion to the amount of colonial growth.

Cultures of the bacteria, including *B. dendroides*, *B. mesentericus*, and *B. mycoides*, were also grown in liquid medium containing 1.0% starch and 0.5% peptone. *B. dendroides* was used in this test, since it is very active in starch hydrolysis. This organism is not so useful for comparison in plate cultures, because of its habit of spreading irregularly in the water film on the surface of agar plates; the width of the enzymic zone from the edge of the growth was about 2 mm. only. In the liquid cultures, *V. amylocella* decomposed the starch in 3 days; *B. dendroides* in 11 days. After 17 days none of the other spore-formers tested had destroyed the starch completely; erythrodextrin was formed only by *B. ruminatus*.

Other Sources of Carbon

As the conversion of starch to glucose by this organism passed through a stage in which iodine gave the colour of erythrodextrin, it was assumed that colourless dextrin would also be a product of the hydrolysis. A medium containing 1% dextrin (Kahlbaum) and 0.1% ammonium chloride was prepared in 100-ml. quantities in Erlenmeyer flasks of 500 ml. capacity. After sterilization, a test was made for reducing value; 10 ml. gave a value equivalent to 4.4 mg. glucose per 100 mg. of dextrin. Eight days after inoculation, glucose was found to be present by the osazone test; the amount in 20 ml. of filtered culture was equivalent to 33 mg. per 100 mg. of the dextrin supplied; the same value was found five days later, by which time the culture was no longer viable.

The action of the organism on glucose (dextrose) was tested with two sources of nitrogen, as it was found that with potassium nitrate the sugar produced from starch was utilized, while with ammonium chloride it accumulated. This was also found to be partly true with dextrose as the sole source of energy. The organism was incubated at 30° C. in flasks containing 20 ml. of mineral salt medium with dextrose (B.D.H. "Analar" quality) in the concentration nearly equivalent to the amount available from 0.2 gm. of starch, *i.e.*, 0.2%, and with 0.2% ammonium chloride. The amount of dextrose recovered, in duplicate tests with the sterile medium, was 95% of the amount added. After two days the amounts of dextrose decomposed were 66% in the potassium nitrate culture and 19% in the ammonium chloride culture. The pH values of the two cultures were 7.2 and 6.0 respectively. On the fifth day all of the sugar had been utilized in the potassium nitrate culture; 57% had been utilized in the ammonium chloride culture, in which the pH value was 5.7. Catalase was present in the culture with potassium

nitrate, the amount of gas produced in the closed arm of a fermentation tube being 33% of the volume of liquid; there was none in the culture with ammonium chloride.

Oxalic acid has been found as a product of the attack on glucose. The organism was inoculated onto plates of glucose agar containing potassium nitrate and calcium carbonate; after a few days octahedral crystals identical with those of calcium oxalate were produced abundantly around the colonies.

Other sources of carbon were also used in synthetic mediums. Lactose, maltose, sucrose, galactose, and raffinose all supplied sources of energy. Sucrose was inverted, the presence of fructose being ascertained by means of Seliwanoff's reagent; typical osazone crystals were also found. A medium with 1% sucrose yielded invert sugar equivalent to 54% of the original sucrose, in eight days; the yield increased to 59% on the 12th day. A medium with 2% sucrose, after 60 days of incubation, yielded invert sugar equivalent to 95% of the original sucrose.

Glucose and fructose were formed from raffinose, but the yield of copper was not more than 9.3 mg. per 100 mg. of the trisaccharide; in a six-days-old culture the osazone crystals resembled those found in the sucrose culture, but crystals of galactosazone could not be found.

In a medium containing 0.2% galactose and 0.1% ammonium chloride, the yield of cuprous oxide on the seventh day was equivalent to 45 to 50 mg. of reducing sugar, calculated as galactose, per 100 mg. of galactose supplied. The amount had not increased on the 10th day. The phenylhydrazine test yielded crystals of galactosazone and brown globular masses of short, blunt-ended crystals that have not been identified. In a medium with 1.0% galactose, the organism utilized only 14% of the sugar in 18 days. The osazone test gave the same results as in the previous test.

Lactose and maltose were decomposed without yielding any trace of glucose; nor did the culture with lactose yield galactose. In 12 days, with ammonium chloride, only 16% of the lactose and 13% of the maltose were utilized. On the other hand, when potassium nitrate replaced the ammonium salt, all of the sugar in a 0.2% maltose medium was decomposed in eight days.

The organism thus appears to be able to decompose these carbohydrates more energetically in the lower concentrations, and when supplied with potassium nitrate in place of ammonium chloride.

The organism grew poorly in a medium containing 0.2% trehalose, and did not produce any glucose.

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SOME BLOOD CHEMICAL CHANGES DURING RECOVERY FROM EXHAUSTIVE MUSCULAR EXERCISE[1]

BY HANS SELYE[2]

Abstract

Experiments in the rat indicate that following a period of intense muscular exercise, marked hyperchloremia develops and is maintained for several days following discontinuation of the exercise. Simultaneously, the red cell concentration decreases and the haematocrit values remain at approximately the same low level at least during the first 72 hr. of the recovery period.

The blood sugar values, which decrease under the influence of muscular exercise, show a marked secondary rise even if the animals are fasted throughout the recovery period.

Introduction

In the course of our experiments on the effect of muscular exercise on the chemical composition of the blood, we noted that a marked increase in blood chlorides and a decrease in blood sugar are characteristic accompaniments of extreme muscular fatigue (3, 4). Haematological studies showed furthermore that after a single period of excessive muscular exercise, the red cell count may decrease considerably and remain low for over 72 hr. (1). It appeared of interest to establish how long the blood chemical changes elicited by muscular exercise would persist and what changes are characteristic of the recovery period.

Methods

Six-months-old female "hooded" rats were used for all the experiments reported in the communication. Their body weights varied between 140 and 224 gm. All experimental animals were forced to perform muscular exercise during four one-hour periods within 24 hr. For this purpose they were placed in drum cages having a diameter of 12 in. and revolving at a speed of 18 to 22 r.p.m. During the exercise period all animals were fasted, because previous experiments have shown that the amount of food ingested may have a considerable influence on the response to muscular exercise, and during the exercise period the amount of food voluntarily taken shows marked individual variations. After the 24 hr. of exercise, the rats were divided into two groups, one of which was fed and the other starved. As fasting in itself influences the haematocrit, blood sugar, and blood chloride values, it seemed advisable to run a special set of not exercised, fasting controls every day, to show the effect of starvation by itself.

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The blood sugar determinations were performed with the Schaffer-Hartmann and Somogyi method. The chlorides were determined in whole blood with the Van Slyke method.

Experimental Results

The results of the experiments are summarized in Fig. 1. Each point on the curves represents the average of three experimental animals killed at the corresponding time. After the completion of the 24-hr. fasting period, the curves representing the exercised animals divide in two, because half of the animals received food during the recovery period, while the other half was fasted. Points on the curves corresponding to the time at which a group of three animals was killed for determinations are marked by squares in the case of the not exercised fasted controls, by dots in the case of the exercised fasted animals, and by crosses in the case of the exercised fed animals. The blood chlorides are expressed as milligrams of sodium chloride per 100 cc. of whole blood and the blood sugars also in milligrams per 100 cc.

The curves in the upper part of Fig. 1 show the average weight loss in grams per rat. It will be seen that although the exercised fasted animals lost a little more weight than the fasted controls, the difference is not very marked. The exercised fed animals, on the other hand, actually gained weight at the end of the experiment so that the curve representing this group declines sharply after the 32nd hr., that is to say, beginning at about 8 hr. after the last exercise period and the administration of food. Between the 72nd and the 96th hr. of the experiment, the curve declines below the zero level of the diagram, since at this time the weight of these animals rose above the initial level.

From the curves representing blood sugar and blood chloride values in the controls, it will be seen that both show a more or less regular and gradual fall, which becomes quite marked at the 96th hr. On the other hand, the blood sugar values in the exercised animals are extremely low at the end of the first 24 hr., but from then on they gradually rise to a level in the fasted animals as high as that of the fed, untreated, normal controls (represented by the point on the ordinate from which all these curves begin). The exercised animals that received food during the recovery period show an actual increase over the initial value at this time. That the initial hypoglycemia caused by muscular exercise may be followed by a secondary hyperglycemic phase even in fasting animals confirms previous observations (5). This is probably due to the fact that exhaustive muscular work elicits a severe "alarm" reaction, characterized by an initial hypoglycemia followed by hyperglycemia. The observation that fasting in itself may elicit a marked "alarm" reaction in thyro-parathyroidectomized rats (2) may explain furthermore why rats deprived of their thyro-parathyroid apparatus respond to continued fasting with an initial hypoglycemia followed by a secondary hyperglycemic phase (3).

The blood chlorides are above normal at the end of the 24-hr. exercise period and remain at this hyperchloremic level for at least 72 hr. from the

beginning of the experiment. There is no constant and significant difference in this respect between the fed and the fasted animals. Since these figures correspond to whole blood chlorides, it would be conceivable that the increase in chloride values is merely due to the decrease in haematocrit values, because

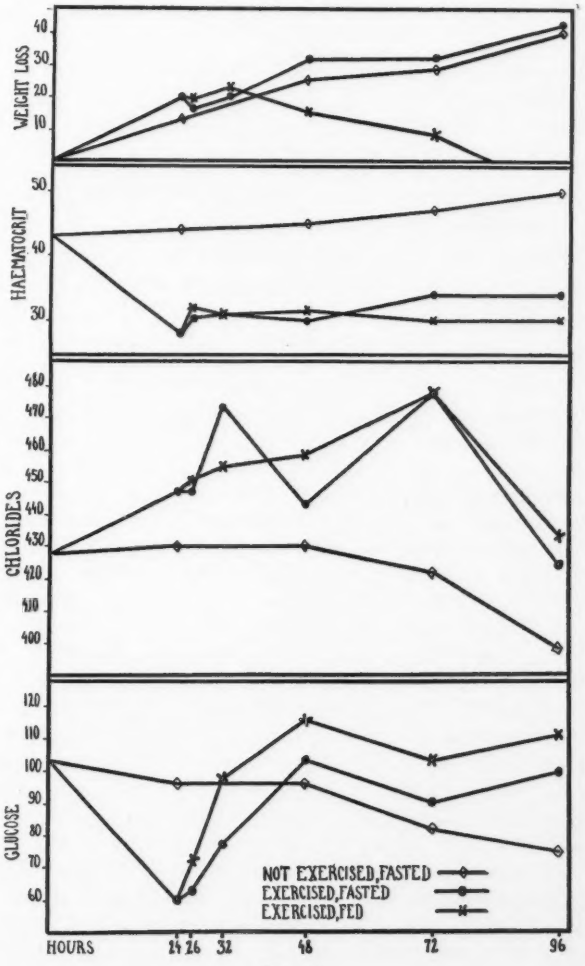

FIG. 1

blood corpuscles are poorer in chlorides than the plasma; consequently, an increase in the relative volume of plasma would necessarily increase the whole blood chloride values unless compensated for by a shift of chlorides from

plasma into tissues. However, Fig. 1 indicates quite clearly that no strict parallelism exists between blood chloride and haematocrit values. For instance, at 72 hr. the blood chlorides are high both in the fed and the fasted exercised animals, while at 96 hr. the chloride concentration of the blood returned to normal in both these groups. On the other hand, the haematocrit values did not change either in the fed or the fasted exercised group at these times.

We must conclude, therefore, that although the rise in blood chlorides is accompanied by, and may partly be due to, a decrease in the red cell concentration, it cannot be regarded simply as a result of the latter. Towards the end of the experiment, the haematocrit values are somewhat higher in the fasted than in the fed group, but this may at least partly be due to the increase in the red cell concentration that is a usual accompaniment of prolonged starvation. In any case, it seems quite evident from the diagrams that the haematocrit values remain at approximately the same low level during the entire length of the 72-hr. recovery period, although the blood chlorides and the body weight return to normal towards the end of the observation period.

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THE SECRETION OF CRYSTALLOIDS AND PROTEIN MATERIAL BY THE PANCREAS IN RESPONSE TO SECRETIN ADMINISTRATION[1]

By S. A. Komarov[2], G. O. Langstroth[3], and D. R. McRae[3]

Abstract

The concentrations of sodium, potassium, calcium, chloride, bicarbonate, and protein and non-protein nitrogen were determined in series of samples of pancreatic juice secreted by dogs in response to (a) constant rate of administration of secretin, (b) varied rate of administration of secretin, and (c) interrupted administration of secretin (rest period, 2 hr.). Spectroscopic as well as chemical methods were used in the analysis of the samples.

The data indicate that the membranes of the pancreatic gland offer little resistance to the passage of simple inorganic ions. The compensatory relation between the bicarbonate and chloride concentrations of the secretion is interpreted on the basis of (a) formation of at least part of the bicarbonate within the secretory cells, and (b) the action of membrane forces, probably of an electrical nature. Absorption spectrum studies indicate either (a) that only one type of absorbing protein is present in the secretion, or (b) that if several types are present, they are always secreted in constant proportions whether they are enzymatically active or not. The interpretation of the protein nitrogen and certain other data are given in a subsequent paper.

A study of the dependence of the composition of a glandular secretion on the conditions of stimulation and on the previous experimental history of the gland may be expected to yield some insight into the nature of the secretory processes. This expectation has been realized for the submaxillary gland (11). The present article describes a study of the secretion of crystalloids and protein material by the pancreas of the dog under various conditions of secretin administration. Three important features of the technique employed in these investigations may be noted here. (a) Only comparatively small samples of secretion were taken from the gland. The interpretation of differences in composition is thereby simplified, since relatively small changes occur in the intra-glandular conditions during short periods of secretory activity. Each of the consecutive samples comprising an experiment was analyzed for its major constituents, as any detailed interpretation of composition differences between widely separated samples requires some knowledge of the behaviour of the gland during the intervening period. These considerations are of the greatest importance in studies of the secretion of protein material. (b) The use of quantitative spectroscopic methods of analysis from both emission and absorption spectra, combined with chemical methods, permitted relatively complete analyses of the comparatively small samples to be made. In addition, absorption spectrum measurements yielded information not easily obtained by other means. (c) A mathematical treatment of the synthesis and secretion of protein material aided considerably in designing experiments to

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show certain characteristic features of the glandular behaviour. This treatment and the interpretation of much of the data of the present article are given in the paper that follows.

The older investigations (reviewed by Roseman (15), Babkin (4, p. 462), and Ball (6, 7)) have indicated that the external secretion of the pancreas originates in a single type of secretory cell, and that it may be evoked by various stimuli. The composition of the secretion varies with the type of stimulus applied. It has been suggested (12) that secretion of protein material is governed solely by the action of the vagus nerves and that secretin is responsible for the secretion of water and crystalloids. This view cannot easily be reconciled with the experimental fact that large quantities of protein material are present in the secretion evoked by secretin even when the action of the vagi is eliminated by section or by administration of large doses of atropine (4, p. 601; also the data of the present article). Furthermore Hammarsten, Ågren and Lagerlöf (10), investigating the effects of injection of purified (but not crystalline) secretin in man, have recently obtained data which they interpret as proving that "secretin stimulates a real time-rate production of enzymes from the pancreatic gland." It therefore appears that secretin itself must be considered to control a mechanism that results in the secretion of protein material as well as of crystalloids and water. For reasons to be stated later the present data are believed to be descriptive of this mechanism.

The more recent investigations (6, 7) have been mainly concerned with the effect of intravenous injections of acids, bases and salts, on the composition of the pancreatic secretion obtained from anaesthetized dogs under secretin administration. There has been no systematic study of the changes in the concentrations and outputs of the inorganic, protein, and non-protein components of the secretion with changes in glandular activity under intravenous injection of secretin.

Experimental Procedure

(a) Physiological technique. Dogs weighing from 5.8 to 17.5 kg. were used throughout the research. They were fasted for 24 hr., and anaesthetized with nembutal. After tracheotomy the vagi were cut in the neck, the abdomen was opened, the small pancreatic duct tied, and the main pancreatic duct cannulated. In order to prevent passage into the intestine of any gastric juice that might be secreted, the stomach was separated from the duodenum by a ligature around the pyloric part of the mucous membrane. The passage of bile into the intestine was prevented by cannulating the bile duct. Artificial respiration was used only in a few cases, when respiration ceased, probably because of an excess of the anaesthetic. A known amount of a secretin solution was introduced every 5 min. into the femoral vein, and the pancreatic juice secreted was collected in graduated test-tubes kept in a beaker containing ice. For the most part the secretion was collected at intervals of 30 min.

The same preparation of secretin, obtained by Still's (17) method from the duodenum and upper jejunum of dogs, was used throughout the work. It corresponded to the preparation designated by Still as "crude" secretin, but was purified further by dissolving in $N/50$ sulphuric acid and precipitating with 10% trichloroacetic acid. The precipitate was extracted with acetone and ether, dried, and preserved in a vacuum desiccator over sulphuric acid in the presence of soda lime. The potency of the preparation did not change to any marked extent during the six months required for the experimental work. The preparation was free from vaso-depressor substances and had only a slight insulin-like effect on the blood sugar.

(b) Analysis of the samples. Total solids and ash were determined in the usual manner, and chlorides by Wilson and Ball's (19) modification of Van Slyke's method. Acid combining power was determined by heating 1 cc. of pancreatic juice with an excess of $N/50$ sulphuric acid (5 or 10 cc.) in a boiling water bath for 10 min., and back titrating with $N/50$ sodium hydroxide to methyl red. It was established in a special set of experiments that the figures so obtained for the acid combining power were virtually identical with those for the carbon dioxide combining power, or alkali reserve, as determined by the volumetric method of Van Slyke and Cullen (13, p. 251). Hence the acid combining power is regarded as representing the bicarbonate content of the secretion. In the determination of non-protein nitrogen, the protein-free filtrates were obtained by precipitating the protein material with five volumes of 9% solution of trichloroacetic acid immediately after the sample was collected. The nitrogen in these filtrates, as well as the total nitrogen in the secretion, was determined by the micro-Kjeldahl method (14, p. 120).

The absorption spectrum of the secretion consisted only of the so-called protein band near λ2800, and end absorption near λ2500. Change in the rate of secretin administration merely altered the strength of the λ2800 absorption. This was measured by a method employing rotating step-sectors and requiring less than 0.3 cc. of secretion for a determination. Knowing the strength of the absorption, an "effective" ϵc value could be calculated from the ordinary absorption law (ϵ = extinction coefficient, c = concentration). In order to give these values some meaning, they were correlated with protein nitrogen data, as will appear later. The secretion was collected and stored on ice for the short time between taking the sample and measuring the absorption. Subsidiary tests showed that under these conditions no appreciable aging or auto-digestive effects occurred.

Sodium, potassium, and calcium were determined by a quantitative spectroscopic method (9).

Results

Results for typical experiments are given in Tables I, II, and III. In the experiment of Table I, the rate of administration of secretin was kept constant throughout, and in that of Table II it was varied. In the experiment of Table III, the administration of secretin was stopped in the middle of the experiment and resumed after a period of 2 hr.

TABLE I

Experiment 5: Dog, male, 15.4 kg. Constant rate of secretin administration.

Sample No.	Vol.†	Na	K	Ca	Cl	ACP	NPN	PN	€c	Solids		
										Total	Org.	Ash
1	8.7	—	—	—	34.8	133	19.3	341	36.0	3.18	2.22	0.96
2	10.0	146	3.3	1.2	30.0	139	22.7	227	24.5	2.46	1.51	0.95
3	10.0	—	—	—	33.2	136	18.5	185	18.7	2.16	1.22	0.94
4	10.0	131	3.3	0.9	38.0	131	18.5	160	15.8	1.99	1.05	0.94
5	10.5	—	—	—	44.8	124	18.5	134	15.6	1.90	0.95	0.95
6	10.3	131	3.1	1.0	48.8	121	18.5	118	13.1	1.81	0.85	0.96
7	10.3	—	—	—	54.4	115	18.5	110	13.1	1.75	0.79	0.96
8	10.0	139	3.3	1.1	58.0	111	18.5	108	13.1	1.72	0.76	0.96

Rate of injection of secretin was 6.1 mg. per 5 min. throughout. The strength of the secretin solution was 2.04 mg. per cc. of secretin in 0.9% sodium chloride plus 0.024 cc. N sulphuric acid. All samples were 30-min. samples.

† *Volume (cc.) of pancreatic juice collected in 30 min.*

TABLE II

Experiment 6: Dog, male, 7.5 kg. Rate of secretin administration varied.

Sample No.	Inj.*	Vol.	Na	K	Ca	Cl	ACP	NPN	PN	€c	Solids		
											Total	Org.	Ash
1	6.0	8.5	—	—	—	86.8	76.4	23.5	164.0	—	2.15	1.22	0.93
2	6.0	10.7	—	—	—	82.0	80.4	21.0	118.0	14.1	1.83	0.89	0.94
3	6.0	10.4	—	—	—	81.6	80.8	21.0	96.0	—	1.69	0.76	0.93
4	6.0	10.6	—	—	—	82.4	81.2	17.6	83.7	—	1.62	0.67	0.95
5	6.0	10.6	143	3.9	3.1	83.2	80.4	17.6	84.3	10.2	1.59	0.64	0.95
6†	1.5	2.0	—	—	—	(93.0)	(67.0)	17.6	55.2	—	—	—	—
7	1.5	4.3	139	3.9	3.3	99.2	58.8	17.6	74.8	8.85	1.50	0.59	0.91
8	1.5	3.7											
9†	6.0	3.4	—	—	—	(93.0)	67.6	19.6	71.7	—	—	—	—
10	6.0	11.6	—	—	—	86.4	74.8	17.9	107.0	—	1.76	0.82	0.94
11	6.0	12.2	—	—	—	86.8	77.6	17.9	96.9	11.4	1.70	0.75	0.95
12†	1.5	3.0	—	—	—	(93.0)	66.8	17.9	72.8	—	—	—	—
13	1.5	4.5	—	—	—	105.0	54.8	19.6	77.3	9.2	1.58	0.65	0.93
14	1.5	3.0											
15†	6.0	3.5	—	—	—	93.6	67.6	19.0	124.0	—	—	—	—
16	6.0	12.2	—	—	—	89.2	74.4	17.9	96.9	11.4	1.74	0.78	0.96
17	6.0	13.0	—	—	—	88.4	75.6	19.6	81.2	—	1.59	0.65	0.94
18	6.0	13.5	113	3.1	3.0	86.4	76.0	17.9	74.5	9.2	1.55	0.62	0.93
19	6.0	13.2	—	—	—	87.2	75.2	17.9	68.9	—	1.50	0.56	0.94
20†	1.0	3.3	—	—	—	—	—	19.6	43.7	—	—	—	—
21	1.0	3.7	143	3.3	3.1	106.0	50.0	19.6	55.4	6.7	1.40	0.48	0.92
22	1.0	2.8											
23	4.0	10.5	—	—	—	88.2	72.0	17.9	67.8	—	1.52	0.57	0.95
24	4.0	9.5	122	3.5	3.7	90.0	72.8	21.8	62.2	8.4	1.47	0.53	0.94
25**	4.0	10.8	170	3.7	3.7	88.4	74.4	20.2	58.8	7.6	1.49	0.54	0.95
26**	4.0	11.0	—	—	—	90.0	76.4	19.6	57.7	—	1.44	0.50	0.94

* *The strengths of the secretin solutions injected were 2 mg. per cc. secretin (Nos. 1–5, 9–11, 15–19, 23–26), and 0.5 mg. per cc. secretin (Nos. 6–8, 12–14, 20–22), in saline plus N/50 sulphuric acid.*

† *These samples were taken to clear the ducts of previous secretion; Nos. 6, 9, and 15 were 10-min. samples; Nos. 12 and 20 were 15-min. samples; remainder were 30-min. samples.*

** *Atropine was injected before No. 25, and again during No. 26.*

TABLE III

Experiment 7: Dog, female, 17.5 kg. Secretin administration stopped in the middle of the experiment, and resumed again after a period of 2 hr.

Sample No.	Inj.*	Vol.	Cl	ACP	NPN	PN	ϵc
1	6.0	4.7	68.0	101	22.7	410	—
2**	6.0	8.8	67.6	104	21.8	297	—
3	8.0	6.4	65.6	106	21.8	174	22.4
4	8.0	6.0	64.8	106	23.5	165	—
5	8.0	6.2	63.6	106	23.5	156	—
6	8.0	6.3	64.8	106	25.2	150	17.0
7†	0.0	0.8	—	—	—	—	—
8†	0.0	0.2	—	—	—	—	—
Rest period, 2 hr.							
9	8.0	5.2	86.4	82.4	31.1	355	(35)
10	8.0	5.9	74.0	98.0	31.1	246	—
11	8.0	5.9	72.8	100	31.1	232	—
12	8.0	6.1	69.6	102	31.1	187	—
13	8.0	6.4	71.2	101	31.1	172	19.4

** The strength of the secretin solution injected was 2 mg. per cc. secretin in saline plus N/50 sulphuric acid.*

*** Sample No. 2 was a 65-min. sample.*

† Samples Nos. 7 and 8 were 5-min. samples taken to ascertain how the rate of flow fell off on stopping the secretin administration. All samples except Nos. 2, 7, and 8 were 30-min. samples.

In these tables, "Inj." denotes the rate of administration of secretin in mg. per 5 min., and "Vol." the volume (cc.) of pancreatic juice collected in each 30-min. period, except where noted. The sodium (Na), potassium (K), calcium (Ca), and chloride (Cl) concentrations and the acid combining power (ACP) are given in milliequivalents per litre, protein and non-protein nitrogen (PN and NPN) in mg. per cent, and total solids, organic matter, and ash in gm. per cent. The ϵc values, calculated per cm. path, are purely relative.

A small sample of secretion was taken immediately after each change in the rate of secretin administration in order to clear the ducts of secretion resulting from the previous stimulation. Such samples (*e.g.*, Nos. 6, 9, 12, 15, and 20 of Table II) may be expected to have a composition intermediate between that of the preceding and that of the following sample, and are of little value in the interpretation of the results.

The following remarks may be made concerning the above data, which are representative of those of other similar experiments.

(a) The composition of the pancreatic juice secreted under a constant rate of secretin administration (Table I). With the exception of the first sample, the rate of secretion of water remained essentially constant throughout the experiment (4 hr.). The concentrations of the investigated metals (sodium, potassium, and calcium) also remained constant. A gradual decrease in the acid combining power during the experiment was compensated for by an increase in the chloride concentration, the sum of the two in milliequivalents per litre remaining constant. The concentration of ash, which is largely

determined by the above-mentioned substances, remained the same throughout the experiment. The concentration of protein nitrogen gradually decreased, the rate of decrease being greatest for the first few samples. The non-protein nitrogen concentration remained unchanged; in some experiments, however, it increased slightly toward the end—*e.g.*, in Experiment 3 (not given here) it rose from 30.8 to 39.2 mg. per cent in $4\frac{1}{2}$ hr.

It was thought that the gradual decrease in the acid combining power and the compensatory relation with the chloride concentration might be due in part to loss of bicarbonates from the system during the experiment, and to the fact that secretin was injected in acid solution. A special experiment in which amounts of sodium bicarbonate equivalent to the bicarbonate loss in the secretion were injected intravenously every half-hour, however, gave results similar to those of experiments in which no replacement was made. The acid combining power fell from 129 to 102, and the chloride concentration rose from 40.7 to 66.2 milliequivalents per litre during the experiment (5 hr.). The results are given in Table IV. The decrease in acid combining power might at first sight be thought to be related to the decrease that occurs in the protein nitrogen concentration. This view is not tenable, however, in the light of such data as given in Table III, which show that the acid combining power of the secretion is not increased after an appreciable period of rest, although a considerable increase takes place in the amount of protein material secreted.

TABLE IV

Experiment 14: Dog, female, 6.5 kg. Constant rate of administration of secretin; bicarbonate lost in secretion replaced by equivalent injections of sodium bicarbonate.

Sample No.	Time	Vol.	Cl	ACP	NPN	PN	$NaHCO_3$ injections		
							Time, p.m.	Vol., cc.	Concent., m. eq./l.
	p.m.								
1	2:00 – 2:10	2.5	—	—	18.9	357.0	—	—	—
2	2:10 – 2:40	7.2	40.7	129	20.2	157.0	—	—	—
3	2:40 – 3:10	7.3	44.0	124	20.2	133.0	—	—	—
4	3:10 – 3:40	7.7	46.9	122	17.6	115.0	3:17	7.3	130
5	3:40 – 4:10	8.7	51.2	118	16.0	102.0	3:50	7.5	125
6	4:10 – 4:40	9.4	54.6	113	17.6	86.6	4:16	8.7	122
7	4:40 – 5:10	9.8	57.2	111	17.6	77.6	4:49	9.4	118
8	5:10 – 5:40	10.3	61.1	107	17.6	72.3	5:19	9.8	113
9	5:40 – 6:10	10.5	62.4	106	18.5	66.6	5:48	10.3	111
10	6:10 – 6:40	10.6	63.9	104	17.6	64.7	6:18	10.5	107
11	6:40 – 7:10	10.5	66.2	102	20.2	61.0	6:47	10.6	106

Rate of injection of secretin was 12 mg. per 5 min. throughout. The strength of the secretin solution was 4 mg. per cc. of secretin in saline containing N/200 sulphuric acid.

(b) The effect of variations in the rate of administration of secretin on the composition of the pancreatic juice (Table II). In Experiment 6 and similar experiments, secretin was injected every 5 min., but the amount injected was varied at the indicated intervals. In the earlier part of the experiment this

was done by varying the strength of the secretin solution, but in the latter part the volume injected was also varied.

The data show that a decrease in the rate of secretin administration results in a decreased rate of flow of water from the gland. It also results in a decrease in the acid combining power and an increase in the chloride concentration of the secretion, the changes being such that the sum of these two factors in milliequivalents per litre remains practically unchanged. The concentrations of the investigated metals, the ash and the non-protein nitrogen are little affected by changes in the rate of secretin administration.

There are wide variations in the behaviour of the protein nitrogen *concentrations* from experiment to experiment, as illustrated by the data of Table V. As will appear later, these variations are probably to be attributed to variations in the operation of the water secretion mechanism.

TABLE V

	Experiment 6	Experiment 8	Experiment 9
Rate of secretin administration per 5 min. per kg. weight reduced:	From 0.8 to 0.2 mg. (*i.e.*, by a factor of 4)	From 0.47 to 0.12 mg. (*i.e.*, by a factor of 4)	From 0.7 to 0.23 mg. (*i.e.*, by a factor of 3)
Decrease in rate of H_2O secretion per 30 min. per kg. weight:	From 1.4 to 0.5 cc (*i.e.*, by a factor of 3)	From 0.7 to 0.09 cc. (*i.e.*, by a factor of 8)	From 0.9 to 0.12 cc. (*i.e.*, by a factor of 7)
Change in protein nitrogen concentration of pancreatic juice:	Increased from 84 to 75 mg. per cent	Increased from 159 to 346 mg. per cent	Increased from 72 to 175 mg. per cent

As in experiments with a constant rate of secretin administration, the protein nitrogen concentration was considerably diminished toward the end of long experiments, while the concentrations of the metals, the ash, and the non-protein nitrogen remained essentially constant.

The data for the last two samples of Table II indicate that the composition of the secretion obtained in response to secretin administration is not appreciably changed on administration of a relatively large dose of atropine (4 mg., and 45 min. later 1 mg., to a dog of 7.5 kg.). Conclusive evidence was obtained by carrying out an experiment in which atropine was administered throughout; the composition of the secretion obtained, and its changes on varying the rate of administration of secretin, were similar to those of experiments in which atropine was not given.

(c) The effect of a period of rest (2 hr.) on the composition of pancreatic juice obtained under a constant rate of secretin administration (Table III). The data show that the concentration of protein nitrogen in the secretion is considerably increased by the 2-hr. period of rest. As will appear in the following paper, this behaviour is probably to be accounted for by a relatively rapid synthesis of protein material within the glandular cells. The increase in the

non-protein nitrogen concentration after the rest period is not necessarily to be explained on the basis of the prolonged interruption of secretory activity, as such an increase is sometimes observed toward the end of experiments with continuous secretory activity. The concentration of chlorides was somewhat higher after the rest period, and the acid combining power somewhat lower, while the rate of secretion of water was not greatly changed. The fact that the acid combining power is not increased after the rest period, while the protein nitrogen concentration is markedly so, has already been referred to in (a).

The Relation between Protein Nitrogen Data and the Absorption Spectrum Measurements

In solutions containing a single type of absorbing protein, the ϵc values obtained from absorption spectrum measurements will be proportional to the protein nitrogen concentrations, providing Beer's law holds. This is the situation, essentially, in the submaxillary saliva of the cat obtained by chorda tympani stimulation (11). If several different types of absorbing protein are present in comparable amounts, however, and if Beer's law holds, the ratio of the observed ϵc value to the protein nitrogen concentration (PN) will be given by

$$\epsilon c/PN = (\epsilon_1 c_1 + \epsilon_2 c_2 + \epsilon_3 c_3 + \ldots .)/(p_1 c_1 + p_2 c_2 + p_3 c_3 + \ldots .)$$

where ϵ_n, c_n and p_n represent respectively the extinction coefficient, the concentration, and the nitrogen fraction of the nth protein. In this equation, $\epsilon c/PN$ becomes constant if $c_1 = \alpha c_2 = \beta c_3$ etc., where α, β, ... are constants. If this is not so, constancy of $\epsilon c/PN$ can be attained only if $\epsilon_1/p_1 = \epsilon_2/p_2 = \epsilon_3/p_3 = \ldots =$ a constant; such a relation between the ϵ's and p's is rather unlikely to occur in complex solutions. Hence if $\epsilon c/PN$ is observed to be constant for solutions containing several types of absorbing protein, the most probable explanation is that the different proteins are always present in constant proportions, no matter what the concentration of total protein may be. Some observed values for the $\epsilon c/PN$ ratio of pancreatic secretion obtained in various experiments (*cf.*, Tables I, II and III) are given in Table VI.

The data show that $\epsilon c/PN$ for samples of pancreatic juice is a constant, even though the total protein content varies over a wide range. In view of the statements made above, this finding indicates that either (a) only one type of absorbing protein is present, or (b) if several types are present, they are always in constant proportions.

It is known that the three principal pancreatic enzymes, namely, trypsin, lipase, and amylase, are secreted in constant proportions (1, 2, 3, 8, 16, 20). If only one type of absorbing protein is present, then presumably it must act as a "carrier" for the different enzymatically active groups. If, however, the enzymes are to be associated with different proteins, the observed constancy of the $\epsilon c/PN$ ratio supports the finding of these investigators. Further-

more, it indicates that not only the different "active" types of protein, but also any inert types present, are always in constant proportions in the secretion. This agrees with the observation that the relative proteolytic power of different samples of pancreatic juice corresponds closely with the relative nitrogen concentrations (5).

TABLE VI

Observed $\epsilon c/PN$ ratios

Exp. No.	*PN* (mg. %)	ϵc	$\epsilon c/PN$	Mean	Exp. No.	*PN* (mg. %)	ϵc	$\epsilon c/PN$	Mean
4	212	23.5	0.111		6	118.0	14.1	0.119	
	183	18.8	0.103			84.3	10.2	0.121	
	172	20.5	0.120			74.8	8.9	0.119	
	137	17.1	0.124			96.9	11.4	0.118	
	146	17.7	0.121			77.3	9.2	0.119	
	127	14.4	0.114			96.9	11.4	0.118	
	153	18.0	0.118	0.116		74.5	9.2	0.123	
						55.4	6.7	0.121	
5	341	36.0	0.106			62.2	8.4	0.134	
	227	24.5	0.108			58.8	7.5	0.129	0.122
	185	18.7	0.102						
	160	15.8	0.098		7	174.0	22.4	0.128	
	134	15.6	0.116			150.0	17.0	0.114	
	118	13.1	0.110			355.0	(35.0)	(0.099)	
	110	13.1	0.120			172.0	19.4	0.113	
	108	13.1	0.122	0.110		225.0	27.1	0.121	0.115

Discussion

The data of the preceding section are believed to describe the operation of a glandular secretion mechanism controlled by secretin alone, since the action of the vagi was eliminated by cutting them or by atropine administration, and the passage of gastric juice and bile into the duodenum was prevented.

The concentrations of sodium, potassium, and calcium in the pancreatic juice secreted under secretin administration were found to be independent of the degree of activity of the gland, as were also the sum of the concentrations of chloride and bicarbonate, and the non-protein nitrogen concentration. This fact suggests that the pancreatic membranes offer little resistance to the passage of simple inorganic ions, since, if it were not so, some dependence might be expected purely on analogy with the behaviour of the submaxillary gland (11). This view is supported by the fact that the concentrations of sodium and potassium in the pancreatic juice are nearly the same as in the blood serum (6, 7). The low concentration of calcium in the secretion as compared with that in the serum is undoubtedly due to the fact that a large part of the calcium in the latter fluid is normally bound in some way to protein molecules and so is in "non-diffusible" form (see (18)). With respect to the results of experiments in which salts are injected into the blood stream (*cf.*, 6, 7), it may be noted that the tendency of some substances at least to

form non-diffusible complexes makes it difficult to interpret such data in terms of membrane permeability for simple inorganic ions.

The acid combining power values given in the tables express very nearly the bicarbonate concentrations. The increase which is observed on increasing the glandular activity (*i.e.*, the secretin injections) is interpreted as due to the increased rate at which carbon dioxide is formed as a metabolic product within the gland. On this basis, a part at least of the bicarbonate has its immediate origin within the secretory cells.

The observed increase in the bicarbonate concentration of the secretion was accompanied by a decrease in the chloride concentration, such that the sum of the two in milliequivalents per litre remained essentially constant. It is significant that in order to obtain a constant sum the concentrations must be expressed in this unit (or in millimoles per litre). It indicates that the number of chloride and bicarbonate ions, or the number of anions per unit volume (other anions are present in concentrations that are small compared to chloride plus bicarbonate), is maintained practically constant. The following interpretation is suggested. A difference in net ionic charge on two sides of a membrane results in an electrical potential difference across it; this potential difference facilitates the passage into the cell of ions of one sign, but hinders that of ions of the other. An increased rate of formation of anions (bicarbonate) within a cell due to increased metabolism results in a change in the potential difference favouring more rapid passage of cations (sodium, potassium, calcium) but retarding the passage of anions (chloride, bicarbonate) from the blood stream or tissue fluids. Since the cations already pass easily through the membrane at the maximum rate (the concentrations in the secretion are comparable with those in the serum), the principal effect is a retarding of the passage of anions, and so appears as a decrease in the chloride concentration of the secretion.

A full discussion of the data on the secretion of protein material is reserved for the following paper. It may be noted here, however, that in some experiments (*e.g.*, No. 6) a decrease in the rate of administration of secretin was accompanied by a *decrease* in the protein nitrogen concentration, while in others (*e.g.*, Nos. 8 and 9) it was accompanied by a considerable *increase* in the protein nitrogen concentration. This difference in behaviour is attributed to a difference in the operation of the water secretion mechanism, since, as will be shown in the following paper, the *output* of protein material exhibits a uniform behaviour.

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